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ADJUSTMENTS OF THE CIRCULATION TO ORTHOSTATIC REACTION AND PHYSICAL EXERCISE DURING THE FIRST TRIMESTER OF PRIMIPREGNANCY

Bo Sandström

From the Department of Obstetrics and Gynaecology (Head. Professor Per Lundström) and the Department of Clinical Physiology (Head. Professor Håkan Linderholm), University of Umeå, Umeå, Sweden

Abstract. The orthostatic pulse reaction, physical working capacity on the bicycle ergometer, total haemoglobin content and the heart volume in the supine position were determined on two different occasions in ten primigravidae, all of whom were in the first trimester of pregnancy at the first examination. The second examination was performed 2 weeks after legal abortion. The orthostatic reaction on standing upright for a period of 8 min was more pronounced during pregnancy than following abortion. The physical working capacity at a pulse rate of 170 was lower during pregnancy than 2 weeks after abortion. There was no difference in the heart volume in the supine position, nor in the total haemoglobin content at the times in question. The circulatory haemodynamics seem to be changed in early pregnancy so as to resemble those of the vasoregulatory asthenia syndrome.

Determinations of physical working capacity orthostatic pulse reaction, heart volume and total amount of haemoglobin have been used to evaluate circulatory function in different disorders (28).

Even in early pregnancy cardiac output (9 20, 30) and renal blood flow (14) are increased. The increase in cardiac output results mainly from an increase in heart rate—10 beats/min increase in the 13th week (22)—and only to a small extent from greater stroke volume (14). The arteriovenous oxygen difference is least during early pregnancy and only late in pregnancy attains non pregnant values (9 30). A pronounced orthostatic pulse reaction during pregnancy has been demonstrated in a number of studies (10, 15, 1 22, 31) and this is greatest during early pregnancy (15). It has been suggested that the diminution of the orthostatic pulse reaction during the course of pregnancy develops simultaneously with the increase in heart rate in the supine position (22).

The physical working capacity expressed as the pulse rate at a given work load, evaluated in tests on the bicycle ergometer remains unchanged during pregnancy according to Robbe (22) and Ihrman (15). In these studies it was not possible to verify any statistically significant decrease during the first trimester of pregnancy. Comparisons were made with values determined 5 weeks after pregnancy (22) and in nonpregnancy (15).

Although the total amount of haemoglobin (15 22) and the heart volume in the supine position increase during pregnancy (15 17 22) they remain essentially unchanged during the first trimester (15 22). Changes of this kind cannot explain the orthostatic pulse reaction during pregnancy.

The orthostatic pulse reaction varies considerably between normal individuals, partly due to variations in height and total blood volume. A linear relationship exists, however between the standing pulse rate and the ratio height/$\sqrt{\text{total haemoglobin}}$ for different groups (28).

An increased orthostatic pulse frequency has been demonstrated in nonpregnant subjects with varicose veins of the leg. After bandaging the legs and following varicotomy the orthostatic pulse frequency decreased (2).

Increased orthostatic pulse frequency is also one feature of the vasoregulatory asthenia syndrome (17). Increased cardiac output as well as a small arterio-venous oxygen difference are parts of the same syndrome.

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Abstract. The orthostatic pulse reaction, physical working capacity on the bicycle ergometer, total haemoglobin content and the heart volume in the supine position were determined on two different occasions in ten primigravidae, all of whom were in the first trimester of pregnancy at the first examination. The second examination was performed 2 weeks after legal abortion. The orthostatic reaction on standing upright for a period of 8 min was more pronounced during pregnancy than following abortion. The physical working capacity at a pulse rate of 170 min was lower during pregnancy than 2 weeks after abortion. There was no difference in the heart volume in the supine position, nor in the total haemoglobin content at the times in question. The circulatory haemodynamics seem to be changed in early pregnancy so as to resemble those of the vasoregulatory asthenia syndrome.

Determinations of physical working capacity orthostatic pulse reaction, heart volume and total amount of haemoglobin has been used to evaluate circulatory function in different disorders (28).

Even in early pregnancy cardiac output (9 20 30), and renal blood flow (14) are increased. The increase in cardiac output results mainly from an increase in heart rate —10 beats/min increase in the 13th week (22)—and only to a small extent from a greater stroke volume (14). The arteriovenous oxygen difference is least during early pregnancy and only late in pregnancy attains non pregnant values (9 30). A pronounced orthostatic pulse reaction during pregnancy has been demonstrated in a number of studies (10, 15, 1 22, 31) and this is greatest during early pregnancy (15). It has been suggested that the diminution of the orthostatic pulse reaction during the course of pregnancy develops simultaneously with the increase in heart rate in the supine position (22).

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The orthostatic pulse reaction varies considerably between normal individuals, partly due to variations in height and total blood volume. A linear relationship exists, however between the standing pulse rate and the ratio height/$\sqrt{\text{total}}$ haemoglobin for different groups (28)

An increased orthostatic pulse frequency has been demonstrated in nonpregnant subjects with varicose veins of the leg. After bandaging the legs and following varicotomy the orthostatic pulse frequency decreased (2).

Increased orthostatic pulse frequency is also one feature of the vasoregulatory asthenia syndrome (1). Increased cardiac output as well as small arterio-venous oxygen difference are parts of the same syndrome.

Table I *Some characteristics of the gravidae taking part in circulation studies during early primipregnancy and 2 weeks after early abortion*

	Pregnancy	After abortion
Age, years	18.0±3 1 (15–24)	
Menarche, years	12.1±0 9 (10–13)	
Height, cm	164.3±4 9 (159–173)	
Weight, kg	53 8±5 1 (45–65)	53.2±5.2 (45–65)
Duration of pregnancy weeks	11 4±1 1 (9–13)	
Time of examination, days after abortion		13.4±0 8 (12 14)

The purpose of the present investigation is to throw more light on the mechanism of circulatory adjustment in early pregnancy This would seem to be possible in a case series in which among other things, the venous volume of the leg is unchanged (25) as in the first trimester of primi pregnancy

MATERIAL

The series comprised ten primigravidae in early pregnancy for whom legal abortions had been granted on psychiatric and sociomedical grounds (24) The blood circulation studies were conducted both before and after abortion. The legal abortions were effected by means of vacuum aspiration (11). Additional data is given in Table I

The patients were told how the trial was to be conducted and gave their full consent.

METHODS

In all cases the examinations were made on two consecutive days so that double determinations of the total haemoglobin would be possible. On the first day the total haemoglobin and heart volume were determined.

The total amount of haemoglobin was determined again on the following day and this was followed by an orthostatic test and a work test on the bicycle ergometer On each day 5 mg diazepam was administered orally before the examination.

The heart rate was measured following 10 min rest in the supine position. The patient was then instructed to stand upright for a period of 8 min with the back of the head leaning lightly against a support. The heart rate and blood pressure were recorded during the orthostatic period. The difference between the heart rate in the supine position and that after 8 min in the upright position was calculated.

The physical working capacity was calculated from the results of an exercise test on the bicycle ergometer (28, 29), during which ECG and heart rate were recorded. Most of the patients could perform the exercise in three periods of 6 min each at 200, 400 and 600 kpm/min. The physical working capacity was defined as the absolute work load coped with by the patient at a heart rate of 170 beats per min and in an approximately steady state, i.e. the heart rate did not increase more than 10 beats from the second to the sixth minute of exercise or not more than 3 beats between the fourth and sixth minute (4 7). The value for the physical working capacity was obtained by extrapolation and interpolation of the approximately linear relationship between heart rate and work load. The heart rate of four subjects during pregnancy and one after abortion did not attain the steady state condition at the highest work load. In these cases the working capacity was estimated from a heart rate of 160 beats per minute by extrapolation and interpolation (28).

The total amount of haemoglobin (THb) was determined by means of the alveolar CO method with slight modifications of the original method (14, 27) Smaller amounts of carbon monoxide were added to the rebreathing system and the CO analyses were performed by the method of Andersson & Dahlström (1). THb determinations were performed on two consecutive days and the error of double determinations was 4.5%

The total blood volume was calculated from the THb and the concentration of haemoglobin in blood from the finger A correction was made for differences between body and peripheral haematocrits using a factor of 0.91 (6)

The supine heart volume was determined by the method of Larsson & Kjellberg (19) and Kjellberg, Rudhe & Sjöstrand (16). The error of a single determination is reported as less than 5% (15 18 22)

RESULTS

The differences between the supine heart rates and the rates following 8 min of standing are significantly greater during early pregnancy than following abortion. The individual differences are indicated in Table II

The orthostatic pulse reaction during early pregnancy is also more pronounced when the heart rate is related to the height of the patient and the total amount of haemoglobin (Fig. 1). The differences in blood pressure at rest and after standing for 8 min show no changes between the period of pregnancy and that following abortion.

The physical working capacity in relation to the circulatory dimensions, the heart volume in the supine position and the total amount of haemoglobin during the first trimester of pregnancy and 2 weeks after abortion is shown on the graphs in

Table II. *Orthostatic pulse reaction*

(1) Supine resting heart rate (beats/min), (2) heart rate after standing upright for 8 min, and change of heart rate = difference between (2) and (1). Physical working capacity in relation to heart volume (HV) and total amount of haemoglobin (THb) during early primipregnancy and after early abortion. OW_{170} = observed working capacity. CW_{170} = working capacity calculated from the regression equations I and II (ref. 13). The difference between OW_{170} and CW_{170} is expressed in per cent (A). Mean ± S.D.

	Pregnancy (pr)	After abortion (apr)	Difference (apr−pr)
Supine 10′ beats/min (1)	73±14	77+18	2.0
Upright 8′ beats/min (2)	126±12	110±18	−12.3
Difference upright–supine (2−1)	47±15	30±14	−17.3
HV ml	546±182	542+181	− 4.0
OW_{170} kpm/min	537±129	632+149	+95.5
CW_{170} kpm/min	637±111	631±94	− 5.8
A	−15.8±15.6	0.3±19.2	16.1
THb g	428±68	418±64	− 9.2
OW_{170} kpm/min	537±129	632±149	95.5
CW_{170} kpm/min	541±109	527±103	−14.8
A	−0.1±20.9	20.8±21.1	22.1

W_{170} = 1.48 HV − 170 S_y = ±148 (eq. I); W_{170} = 1.60 THb − 141 S_y = ±130 (eq. II).
T... patients could not stand upright for 8 min during pregnancy and are therefore excluded from the *t*-test.
These function underlines the orthostatic instability during early pregnancy.
(0.01 < P < 0.05), (0.001 < P < 0.01)

Figs. 2 and 3. The percentage deviation (A %) of the observed working capacity from the working capacity calculated from the relationships W_{170} = 1.48 HV − 170 and W_{170} = 1.60 THb − 141 (13) is given in Table II. The physical working capacity is lower during pregnancy than 2 weeks after abortion. The difference becomes more pronounced when the observed physical working capacity in each group is related to the working capacity calculated as indicated above.

The heart volume in the supine position as well as the total haemoglobin and total blood volume remain unchanged during the first trimester of pregnancy as compared with those parameters 2 weeks after abortion.

DISCUSSION

During the first trimester of pregnancy the circulatory system of the pregnant woman undergoes substantial change which does not seem to be commensurable with actual requirements at this time. When the change in heart rate with change in posture from lying to standing for 8 min is calculated, an increase in pulse frequency during pregnancy becomes noticeable, which agrees with earlier findings (15).

The decrease in physical working capacity dur

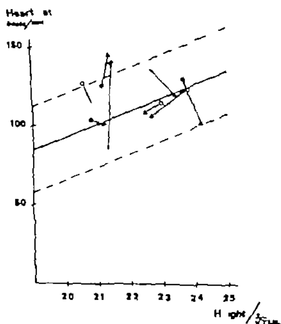


Fig. 1. Heart rate in beats per minute after standing for 8 min in relation to body height divided by cube root of THb. The straight line represents the normal regression line with the equation Y = 8.3 ... 72.8 S = ± 13.6 (12). Dashed lines indicate variation ± 2 S_y (standard error of estimate). Empty circles indicate pregnancy and filled triangles 2 weeks after early abortion.

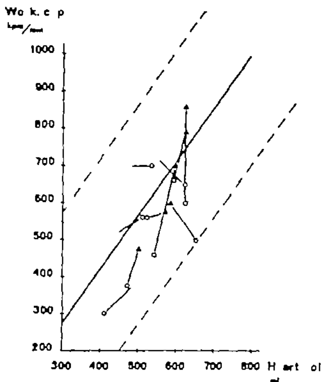


Fig 2 The relationship between physical working capacity t a heart rate of 170 beats per minute (W_{170}) and heart volume (*HV*) during early pregnancy (empty circles) and weeks afte early abortion (solid triangles). Regression line ± S

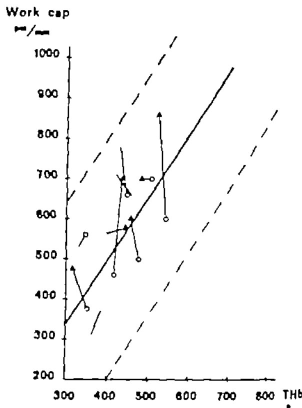


Fig 3 The relationship between physical working capacity at a heart rate of 170 beats per minute (W_{170}) and the amount f total haemoglobin (THb) during early pregnancy (empty circles) and 2 weeks after early abortion (solid triangles) Regression line ± S_e

ing early pregnancy is not quite in agreement with the findings of Robbe (2.) or Ihrman (15). However the latter author reported a tendency to higher pulse frequency during early pregnancy —at a work load of 600 kpm/min—than during nonpregnancy (5 beats per minute) In the present study the mean age of the subjects is about 10 years lower and the women are all primigravidae which may explain some of the difference.

Changes of a type similar to those found in the present investigation were observed by Arenander (2) and Arenander & Carlsten (3) on nonpregnant women with varices of the leg. Similarly this reaction pattern could during early pregnancy be explained by an accumulation of blood in dilated leg veins. However it has not been possible to demonstrate plethysmographically an increased venous volume in the lower legs during normal primipregnancy (25). Nor do phlebographic ex aminations of the leg veins in the upright position during the first half of primipregnancy indicate increased dilatation of the veins compared with the nonpregnant state (23) As vein diameter and venous volume of the leg are essentially un changed during early pregnancy (23 .5) a m pronounced orthostatic blood volume redistri tion than in normal subjects does not seem to plain the orthostatic pulse reaction The lo cardiac output in relation to oxygen consumpti combined with a lower physical working capaci and a high orthostatic pulse frequency indica circulatory changes similar to those of the vas regulatory asthenia (VA) syndrome (1) If duri early pregnancy a hyperkinetic circulation present also in the legs this should mainly affe nonmuscular tissue (5 8 4).

REFERENCES

1 Andersson, T & Dahlström, H Scan Tools (1958.

. A enander E. Acta Chir Scand, S ppl .60 196

3 Arenander E & Carlsten, A Acta Chi Sc nd 11 570, 1961

4 Bevegård, S H lmgren, A & J nss B Act Physiol Scand 49 79 1960.

5 Carlsson, L., Persson B. & Zetterquist, S. Acta Med Scand 172, 389 1962.
6 Eklund, L-G., Scand J Clin Lab Invest, Suppl. 86 17 1965
7 Forsberg, C. & Schmalensee, G Acta Physiol Scand 73 435, 1968.
8 Graf, K. & Strom, G: Arch Kreislaufforsch 50 231 1966.
9 Hamilton, H F H J Obstet Gynaec Brit Emp 56 548, 1949
10 Hansen, R Klin Wschr 21 241, 1942.
11 Holmberg, N G. & Sandstrom, B Lecture at Congress of the Swedish Med Assoc, Stockholm, 1970 Acta Obstet Gynaec Scand 51 143, 1972.
12 Holmgren, A Jonsson, B Levander Mal, Linderholm, H Sjostrand, T & Ström, G Acta Med Scand 158, 413 1957
13 Holmgren, A Jonsson, B Levander Mal, Linderholm, H Mossfeldt, F Sjöstrand, T & Strom, G.: Acta Med Scand 165 89 1959
14 Hytten, F E & Leitch, Isabella: The Physiology of Human pregnancy Blackwell Scientific Publications, Oxford, 1964 1971.
15 Ihrman, K A Clinical and Physiological Study of Pregnancy in Material from Northern Sweden. Uppsala, 1960.
16 Kjellberg, S R Rudhe, U & Sjostrand, T Acta Radiol (Stockh) 31 113 1949
17 Kjellberg, S R, Lönroth, H Rudhe, U. & Sjöstrand, T Acta Med Scand 138, 421 1950.
18 Kjellberg, S. R., Lönroth, H. & Rudhe, U Acta Radiol (Stockh) 35 413, 1951.
19. Larsson, H. & Kjellberg, S. R., Acta Radiol (Stockh) 29 159 1948.
20 Palmer A. J & Walker A. H. C., J Obstet Gynaec Brit Emp 56. 537 1949
21 Pyörälä, T Acta Obstet Gynec Scand 45 Suppl. 5, 1966.
22. Robb.., Hjordis: Circulatory dimensions and circulatory function during and after pregnancy physiological study in iron-treated women, in anaemic cases and in patients with heart disease. Stockholm, 1959
23. Sandström, B. & Löwegren, L. Acta Obstet Gynec Scand 49 375 1970.
24 Sandström, B. Acta Obstet Gynec Scand 5.. 199 1973
25 —Acta Obstet Gynec Scand 52. 289 1973
26. Sjöstrand, T Acta Med Scand, Suppl. 196 687 1947
27 —Acta Physiol Scand 16. 211 1948.
28. — Clinical Physiology Sv Bokförlaget Bonniers, Stockholm, 1967
29 Wahlund, H. Acta Med Scand, Suppl. 315 1948.
30. Werko, L. Acta Obstet Gynec Scand 33 162, 1954
31. Widlund, G. Acta Obstet Gynec Scand 25 Suppl. 1 1945

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THE EFFECT OF RADIUM ON BLOOD FLOW IN THE HUMAN UTERINE CERVIX MEASURED BY LOCAL HYDROGEN CLEARANCE

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Abstract. Cervical blood flow was measured by the local hydrogen gas clearance method in 15 patients with untreated carcinoma of the uterine cervix and in 16 patients six to eight weeks after radium irradiation. Mean cervical blood flow was 73.5 ml/min/100 g in the untreated group and 92.4 ml/min/100 g in the treated group.

Some malignant epithelial tumours including carcinoma of the cervix uteri, are known to contain areas that vary in their degree of oxygenation (9). The oxygen is supplied through a system of more or less well-developed capillary blood vessels (8, 9). It seems reasonable to believe that external factors altering the flow in the tumour tissue also alter the flow in the underlying fibro-muscular tissue of the uterine cervix. Ionizing irradiation is known to induce an initial hyperaemia followed later by a reduced vascularization (6). Rubin & Casarett (11) demonstrated by the use of correlative microangiographic and histological techniques a so-called supervascularization phenomenon in irradiated regressing tumours in rats. In carcinoma of the human uterine cervix Bergsjø (2) observed indication of "an increasingly better blood supply" during the initial phase of fractionated radiation therapy. No direct measurements of blood flow through the cervix were performed. The aim of the present study was to measure the effect of radium irradiation on cervical blood flow.

METHODS

Local blood flow in one, two or three different areas in the uterine cervix was recorded with the hydrogen gas clearance method (1, 7). Patients with untreated early carcinoma of the cervix had recording electrodes inserted both through the surface of the tumour and through the adjacent mucous membrane. Every attempt was made to place the electrodes in the fibro-muscular tissue of the cervix, and not in the tumour tissue. After radium application the electrodes were again inserted in the area where the carcinoma had been located. Repeated measurements with the electrodes undisturbed were as a rule performed at intervals of about 20 min. Only desaturation curves showing an exponential washout with at least 80% desaturation were used for flow calculation.

Radium treatment was given by a modified Paris method (4). The total dose varied between 6 000 mg/h and 7 200 mg/h, delivering approximately 7 000 rad to point A and 1 000 to 1 500 rad to point B in the course of ten days. As defined by Tod & Meredith (12) point A lies 2 cm lateral to the central canal of the uterus and 2 cm above the mucous membrane of the lateral fornix in the axis of the uterus. Point B lies 3 cm lateral to point A at the same level.

Measurements of cervical blood flow were performed from 43 to 65 days after completion of radium application. This time interval was determined by the treatment schedule for Stage Ib and early Stage II lesions used in the Norwegian Radium Hospital. The routine is to give primary radium therapy followed six to eight weeks later by radical hysterectomy and pelvic lymphadenectomy.

The Wilcoxon rank test and the Student' *t*-test were used to evaluate the significance of the difference between the observed blood flow values before and after irradiation. The blood flow values in the tables are given ± S.E.

MATERIAL

Cervical blood flow was measured in 15 patients before and in 16 patients after radium treatment. In three of the patients (Nos. 1, 2 and 3) measurements were performed both before and after irradiation.

Stage distribution in the material (International Classification System) is shown in Tables I and II. A total of 22 patients belonged to clinical stage I and 6 to stage II. In the untreated group the age of the patients varied between 32 and 67 years, in the treated group between 25 and 61 years, with mean age 43.7 and 42.3 years

Table II *Cervical blood flow in patients after radium therapy of carcinoma of the cervix*

Pat. no	Age (y.)	No. of pregnancies	Clinical stage	Days after completion of therapy	First measurement ml/min 100 g: Each electrode	First measurement: Mean	Second measurement ml/min 100 g: Each electrode	Second measurement: Mean
1	45	2	Ib	59	77.0 84.0 64.0	75.7		
2	47	3	Ib	63	179.8	129.8	123.3	123.3
3	43		Ib	58	52.3	52.3	43.3	43.3
16	32	2	Ib	61	81.5 92.4 57.8	77.2	99.0 118.0 52.3	89.8
17	31	4	Ib	45	136.3 118.9 109.5	121.6	134.6 103.9 94.5	111.0
18	46	2	II	58	69.3 94.0	81.7	67.6 84.0	75.8
19	49	5	Ib	56	138.6 133.8	136.2	78.5	78.5
20	40	2	Ib	43	46.2	46.2		
21	61	5	Ib	46	35.6 53.3	44.5		
22	33	2	Ib	48	92.4 69.3	80.9	104.1 69.3	86.7
23	35	3	Ib	46	134.0 134.0	134.0	112.3 124.2	118.3
24	40	4	Ib	51	99.0 138.6	118.8	99.0 138.6	118.8
25	46	1	Ib	42	117.1 103.9	110.5	99.0 100.1	99.6
26	44	2	Ib	43	154.0 100.9	127.5	143.3 101.3	122.4
27	44	5	Ib	48	67.5	67.5	67.5	67.5
28	49	3	Ib	44	66.0 81.5	73.8	57.8 102.7	80.3
Mean ± S.E.						92.4±8.1		
Mean for patients having first and second measurements						100.9		91.5
Mean for patients 1, 2 and 3 having measurements before and after radium therapy						85.9		

As evident from Tables I and II the recorded flow in some cases was twice as high in one area compared with another when simultaneous recordings were made in the same cervix. In most cases the observed flow values in the same cervix showed good correlation. A variance analysis showed that the flow scatter was about five times higher between patients than between electrodes in the same cervix both in the untreated and the treated groups.

Mean blood flow in the group of patients in which measurements were performed after radium treatment was found to be 122.4 % of the mean flow in the untreated patient group. Tested by the Wilcoxon test ($p > 0.10$) and the Student's t-test ($p = 0.16$) there was no significant difference between the two groups (Table I and Table II). It must be stressed, however, that the series is small for such a statistical evaluation. Furthermore each of the three patients in whom recordings were made both before and after treatment (Nos 1, 2 and 3 in Tables I and II) showed an increased flow after treatment with a mean flow of 124.5 % of that recorded before radium therapy. The two oldest patients in the series (Nos 5 and 21) both had a low cervical blood flow.

DISCUSSION

No method for measurement of human uterine cervical blood flow repeated precisely at the same area with intervals of weeks is as yet available.

Table I *Cervical blood flow in patients with untreated carcinoma of the cervix*

Pat. no	Age (y)	N of pregnancies	Clinical stage	First measurement ml/min 100 g Each electrode	Mean	Second measurement ml/min 100 g Each electrode	Mean
1	45	2	I *b*	74.9 59 0 53.3	62.4	60 6 57 8 37 0	51 8
	47	3	I *b*	86 6 102.7	94 7	81.5 92.4	87.0
3	45	2	I *b*	55 4 47 8 46..	49 8	53 3 36.5 37.5	42.4
4	33	2	II *b*	69.3 57 8	63 6	55 4 46.3	50 8
5	67	2	II	39 0 42.0	40.5		
6	44	2	I *b*	53.3 103 4 96.3	84 3		
7	44	2	I *b*	138 6	138.6		
8	39	0	I *b*	84 0	84 0	81.5	81.5
9	48	5	II *b*	72.0 48.6	60.3	79.. 44 0	61 6
10	39	2	I *a*	77 0	77 0	77.0	77 0
11	50	3	I *b*	47 8 99 0	73 4	47 8 99 0	73 4
12	33	0	II *b*	31.5 43.3	37 4		
13	35	4	II *a*	51.3 77 0	64.2	47 8 77 0	62.4
14	54	2	I *b*	64 8 53 3	59 1	49.5	49.5
15	32	3	I *b*	126 0 160.0	143 0	115 5 119 1	117.3
Mean ± S E.					75.5 ± 8.0		
Mean for patients having first and second measurements					75 6		68 6
Mean for patients 1 2 and 3 having measurements before and after radium therapy					69 0		

respectively Only two patients had never been pregnant. Fou patients had passed the menopause.

Gynaecological examination and measurement of the uterine cavity showed that all uteri were f normal size.

RESULTS

In 15 patients with untreated carcinoma of the cervix local cervical blood flow was recorded at 30 different sites which gave a mean flow rate of 75.5 ml/min 100 g with a range of 40 5 to 143 ml/min 100 g (Table I) Repeated measurements were obtained in 11 cases with the elec trodes in the same position Mean flow rate at 21 electrode sites was 73 7 ml/min 100 g at the first measurement and 66 5 ml/min 100 g at the sec ond which indicates a mean decrease to 90.2 of the first measurement. Only once was bloc flow found to be sligthly higher at the secon measurement (Table I Patient 9)

Forty three to sixty five days after radium trea ment cervical blood flow at 31 different sites i 16 patients showed an average of 92.4 ml/mi 100 g with range 44 5 to 136 2 ml/min 100 (Table II) In this group repeated measurement from 24 electrodes in 13 patients were obtained The mean blood flow at the first and secon measurement was 101 6 ml min 100 g and 96 ml/min 100 g respectively a reduction to 95 1 of the first recordings. Blood flow was foun slightly increased at five out of twenty four elec trode sites at the second measurement (Table II Patients 16 .2, 26 and 28).

Table II. *Cervical blood flow in patients after radium therapy of carcinoma of the cervix*

Pat. no	Age (y.)	N of pregnancies	Clinical stage	Days after completion of therapy	First measurement ml/min 100 g		Second measurement ml/min 100 g	
					Each electrode	Mean	Each electrode	Mean
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16	32	2	Ib	61	81.5 92.4 57.8	77.2	99.0 118.0 52.3	89.8
17	31	4	Ib	45	134.3 118.9 109.5	121.6	134.6 103.9 94.5	111.0
18	46	2	II	58	69.3 94.0	81.7	67.6 84.0	75.8
19	49	5	Ib	56	138.6 133.8	136.2	78.5	78.5
20	40	2	Ib	43	46.2	46.2		
21	61	5	Ib	46	35.6 53.3	44.5		
22	33	2	Ib	48	92.4 69.3	80.9	104.1 69.3	86.7
23	25	3	Ib	46	134.0 134.0	134.0	112.3 124.2	118.3
24	40	4	Ib	51	99.0 138.6	118.8	99.0 138.6	118.8
25	46	1	Ib	62	117.1 103.9	110.5	99.0 100.1	99.6
26	44	2	Ib	43	154.0 100.9	127.5	143.5 101.3	122.4
27	44	5	Ib	48	67.5	67.5	67.5	67.5
28	49	3	Ib	44	66.0 81.5	73.8	57.8 102.7	80.3
Mean ± S.E.						92.4 ± 8.1		
Mean for patients having first and second measurements						100.9		93.5
Mean for patients 1, 2 and 3 having measurements before and after radium therapy						85.9		

As evident from Tables I and II, the recorded flow in some cases was twice as high in one area compared with another when simultaneous recordings were made in the same cervix. In most cases the observed flow values in the same cervix showed good correlation. A variance analysis showed that the flow scatter was about five times higher between patients than between electrodes in the same cervix both in the untreated and the treated groups.

Mean blood flow in the group of patients in which measurements were performed after radium treatment was found to be 122.4% of the mean flow in the untreated patient group. Tested by the Wilcoxon test ($p > 0.10$) and the Student's t-test ($p = 0.16$) there was no significant difference between the two groups (Table I and Table II). It must be stressed, however, that the series is small for such a statistical evaluation. Furthermore each of the three patients in whom recordings were made both before and after treatment (Nos. 1, 2 and 3 in Tables I and II) showed an increased flow after treatment with a mean flow of 124.5% of that recorded before radium therapy. The two oldest patients in the series (Nos. 5 and 21) both had low cervical blood flow.

DISCUSSION

No method for measurement of human uterine cervical blood flow repeated precisely at the same area with intervals of weeks is as yet available.

The method used in the present study permits repeated measurements with the electrodes in the same position for periods of some hours. In agreement with previous observations (7) the scatter of flow between patients was higher than the scatter between simultaneous measurements in the same cervix. The recordings showed a similar tendency to increased flow after radium treatment both by repeated measurements in the same cervix and in the groups of different patients.

Comparable values could only be obtained when blood flow was measured in the fibro-muscular tissue of the cervix, because the tumour with its sign of infection in most cases had disappeared after radium therapy Both the depth of insertion and the insertion of the electrodes through the soft tumour into a firmer tissue, indicated that the electrodes were positioned in the fibro-muscular tissue of the cervix even if the exact localisation of the electrode tips could not be determined

The flow scatter between areas in the same cervix might be due to infiltration and infection of the tumour tissue, but a similar variation was also observed in patients not suffering from cervical carcinoma (7). As discussed before (7) changes in the cervical connective tissue after childbirth and as a result of pathological conditions might be the reason for the variations. In the radium-treated group various amounts of fibrosis might increase the flow differences.

The variability of flow between patients could not be correlated with parity depth of electrode insertion or stage of the carcinoma. Compared with a reported mean flow of 73 4 ml/min 100 g in 13 patients of the same age suffering from metrorrhagia (7) carcinoma of the cervix in its earlier stages seems not to influence the mean blood flow in the fibro-muscular tissue of the cervix.

Radium irradiation lowers ovarian hormonal function and induces a premature menopause. The cervical tissue undergoes fibrosis reduced vascularization and atrophy but before these retrogressive changes occur a transient phase of hyperaemia develops in the cervix. This hyperaemia might be due to vasodilatation (6) to shrinkage of the tumour tissue (2) which in turn results in a relative increase in the number of capillaries, to development of new vessels, or possibly a combination of all these factors. The present study indicates that increased blood flow per weight unit of tissue may be partly responsible for the hyperaemia This is in agreement with the assumption of Bergsjø (2) based on colposcopic and colpo-photometric studies of the intercapillary distance and the diameter of the vessels in carcinoma of the uterine cervix.

During the early phase of external irradiation, Bergsjø & Evans (3) found an increased level of oxygenation in cervical carcinoma. Their measurements were performed with a micro-electrode introduced into the tumour tissue Cater & Silver (5) also observed that the oxygen tension in the tissue adjacent to the tumour rose immediately after a course of irradiation Both observations might be explained by destruction of cells resulting in lowered oxygen consumption However the present measurements of blood flow in the fibro-muscular tissue indicate that the increased oxygenation after irradiation might rather be due to increased cervical blood flow per gram of tissue.

Though the blood flow measurements relate only to cervical tissue, it is reasonable to deduce that wound healing after surgical treatment will be satisfactory six to eight weeks after radium irradiation at least as far as blood flow may be responsible for healing. This is well supported by the results of a large series of radical hysterectomies (10) performed after that time interval in the Norwegian Radium Hospital

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REFERENCES

1 Aukland, K Bower B F & Berliner R. W Measurement of local blood flow with hydrogen gas. Circ Res *14* 164–187 1964
2. Bergsjø, P Radiation-induced early changes in size and vascularity of cervical carcinoma. Acta Radiol, Suppl. 274 1968.
3 Bergsjø, P & Evans, J C. Oxygen tension of cervical carcinoma during the early phase of external irradiation. Scand J Clin Lab Invest *27* 71–82, 1971
4 Bergsjø, P & Kristiansen, P Treatment method and dose distribution in radiotherapy of carcinoma of the cervix. Acta Radiol Ther *7* 181 192, 1968
5 Cater D B. & Silver I. A. Quantitative measurements of oxygen tension in normal tissue and in the tumours of patients *before and after radiotherapy* Acta Radiol *53* 233–256, 1960.

6. Devik, F. A study of the local roentgen reaction on the skin of mice, with special reference to the vascular effects. Acta Radiol, Suppl. 119. 1955.
7. Klingenberg, I. & Aukland, K. Measurement of human uterine cervical blood flow by local hydrogen gas clearance. Acta Obstet Gynec Scand 48, 455–469. 1969.
8. Kolier, O. The Vascular Patterns of the Uterine Cervix. Universitetsforlaget, Oslo, 1963.
9. Kolstad, P. Vascularization, Oxygen Tension, and Radiocurability in Cancer of the Cervix. Universitetsforlaget, Oslo, 1964.
10. — Lymph node metastases in cancer of the cervix Stage I b. Aust N Z J Obstet Gynaec 8, 107–116, 1968.
11. Rubin, Ph. & Casarett, G. Microcirculation of tumors. Part II. The supervascularized state of irradiated regressing tumors. Clin Radiol 17, 346–355. 1966.
12. Tod, Margaret & Meredith, W. J. Treatment of cancer of the cervix uteri—a revised "Manchester method". Brit J Radiol 26, 252–257. 1953.

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REFERENCES

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3. Bergsjø, P. & Evans, J. C. Oxygen tension of cervical carcinoma during the early phase of external irradiation. Scand J Clin Lab Invest 27 71–82, 1971
4. Bergsjø, P. & Kristiansen, P. Treatment method and dose distribution in radiotherapy of carcinoma of the cervix. Acta Radiol Ther 7 181–192, 1968.
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SERUM TRIGLYCERIDE IN WOMEN WITH AND WITHOUT A PREDISPOSITION TO DIABETES DURING SHORT TERM ADMINISTRATION OF A COMBINED ORAL CONTRACEPTIVE

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Abstract A group of 17 healthy women, of whom 9 had predisposition to diabetes, were studied for five consecutive menstrual cycles with regard to the effect of an oral combined contraceptive containing 0 5 mg norgestrel and 0 05 mg ethinylestradiol (Eugynon®) The fasting concentration in the serum of triglycerides, free fatty acids and insulin together with the response values during i glucose tolerance for free fatty acids and insulin were estimated The tests were performed at midcycle in the cycle prior to, in two cycles during and in the second cycle after cessation of the drug administration The drug was administered daily from the fifth to twentyfifth day of the cycle in the two cycles. Thus each subject served as her own control Statistical analysis of the results showed significant increase in the fasting values of the serum triglycerides during hormonal administration in those predisposed to diabetes, but unchanged values in those not so predisposed. The fasting values of the serum free fatty acids were for the series as whole, significantly lower in the second cycle of administration There was no significant difference with regard to the predisposition to diabetes, but tendency to unchanged values in those predisposed to diabetes and lower values in those not predisposed The fasting values of the serum insulin showed no changes and no difference with regard to predisposition to diabetes The response to glucose injection showed, for the free fatty acids, no significant changes during hormonal administration, but the fasting values also had tendency to unchanged values for those predisposed and lower values for those not predisposed to diabetes, during hormonal administration The response values of serum insulin were, for the series as whole, significantly higher during hormonal administration, those predisposed to diabetes showed 60 min after glucose injection significantly higher values than those not so predisposed during hormonal administration The results are discussed and compared to the findings of other investigators. Possible modes of action with regard to the increase in triglycerides in those predisposed to diabetes are discussed The study indicates, but does not prove, that in women predisposed to diabetes there is an increased risk of the development of hypertriglyceridaemia and consequent disease with the administration of the oral contraceptive studied

Experience during recent years with regard to the relationship between increased concentrations of serum lipids and the development of arteriosclerotic disease (4, 17) has brought about the fear that the widespread use of hormonal contraceptives, which may affect lipid metabolism (1 10 27), will increase the risk of arteriosclerotic disease (3 6, 9 10). It has not been possible in studies performed to date to demonstrate with certainty that the use of an oral contraceptive increases the risk of developing coronary disease (3)

Oral contraceptives of the combined type have to date been superior to other hormonal contraceptives, with regard to high contraceptive effect and low frequency of bleeding disturbances. Therefore it must be presumed that, despite the higher frequency of metabolic side-effects than occurs with the sequential preparations and the pure gestagens, they will continue to be used in future. It is therefore of importance that such combinations and dosages of oestrogen and gestagen derivatives are chosen that the metabolic changes, including changes in the serum lipid, are as small as possible (6, 7) Similarly it is of importance to exclude individuals who, owing to predisposition, are particularly exposed to the development of metabolic changes during the use of combined oral contraceptive (14)

The object of the present study is to determine whether short term administration of one

these showed no significant changes in the serum triglycerides during the period of administration for the series as a whole. When divided into the two groups D and N those predisposed to diabetes were found to have higher values during administration than those not so predisposed. Variance analysis showed a statistically significant difference with regard to the diabetic predisposition ($p < 0.001$).

The results of the free fatty acids estimations are given in Fig. 3 as the mean values for the series as a whole and for each of the groups D and N. The figure consists of four diagrams, one for each of the four sets of tests, corresponding to the four glucose tolerance tests. With regard to the fasting values, it was found for the series as a whole that there were lower values in the second hormone administration cycle ($p < 0.02$). When divided into groups it appears that those predisposed to diabetes had unchanged values in all four tests, while those not so predisposed had lower values in the second hormone administration cycle. However variance analysis showed

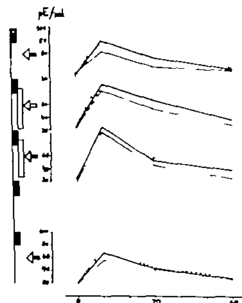


Fig. 4. Fasting insulin and insulin response to 1 glucose injection. (Construction of the figure and symbols are the same as in Fig. 3.)

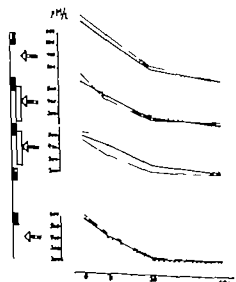


Fig. 3. Fasting free fatty acids and free fatty acid response to 1 glucose injection. The figure consists of four diagrams, placed one above the other, one for each of the four tests. (Symbols are the same as in Fig. 2.)

no statistically significant difference between the two groups.

With regard to the response values to the free fatty acids after glucose injection it was found that there were no significant changes for the series as whole during hormone administration. With regard to diabetes there is pparently tendency to those not predisposed having lower values during hormone administration, particularly in the second cycle, while the values for those predisposed to diabetes were unchanged. However this could not be confirmed by variance analysis.

The results of the serum insulin tests are shown in Fig. 4. The mean values for the whole series and for each of the groups D and N are given in four diagrams, one for each of the four sets of tests, corresponding to the four glucose tolerance tests. The statistical analysis of the results of the fasting insulin tests showed for the series as whole no changes during the period under study. Variance analysis showed no difference between those predisposed to diabetes and those not so predisposed. The series as a whole showed

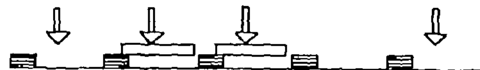


Fig 1 Plan of the investigation Hatched areas = menstruation clear areas = hormone administration arrows = tests.

of the combined contraceptives frequently used today has an effect on the serum concentrations of triglycerides and free fatty acids, also whether the presence of a predisposition to diabetes is of importance in this respect. At the same time determinations were made of parameters of carbohydrate metabolism on the test subjects and these results have been published earlier (19).

MATERIAL AND METHODS

The series consists of 17 women who consulted us with a view to commencing oral contraception. Their ages varied between 20 and 39 years, average 27 years. Nine of the women were classified as being predisposed to diabetes as they fulfilled one or more of the following criteria Diabetic glucose metabolism during an earlier pregnancy the birth of a child ≥4 500 g, increase in weight ≥20 0 kg during an earlier pregnancy hereditary predisposition to diabetes (group D). The other eight were without such a predisposition (group N) All the women were healthy and had no signs or history of any endocrinological disease and none of them were under treatment with any kind of drug. Before entering the trial they were all instructed to maintain their normal diet Those admitted to the investigation had regular menstruation and a minimum of 3 months had passed since their last pregnancy Apart from the above no selection was made for the study There was no statistical difference between groups D and N with regard to age and parity

The women were studied for a duration of five menstrual cycles, tests were performed four times during the period of observation, namely at midcycle in cycles 1, 3 and 5 (see Fig. 1). An oral contraceptive (0.5 mg Dl norgestrel and 0.05 mg ethinyloestradiol) were given dai from the 5th to 25th days of cycles 2 and 3

The tests included the determination of fasting valu of the serum triglycerides, serum free fatty acids a serum insulin, together with response alues for fr fatty acids and insulin following i.v glucose injectio The tests were carried out in the morning after t women had fasted for 12 hours. For the fasting te venous blood was withdrawn from a cubital vein a this was followed by the injection of 50 ml of 50% glucose i.v In the following hour during which th subject remained at rest venous blood wa withdraw for the determination of response values at 10, 30 a 60 min.

The serum triglycerides and free fatty acids were de termined by the Department of Physiology Odense Uni versity Odense (according to the method of Laurell a Laurell-Tibblin respectively). The determination of seru insulin was carried out by the Department of Pharmaco logy University of Lund, S eden (according to the metho of Hedin)

The statistical analysis of the material, which was car ried out t the EDB Centre Odense U iversity Hospital included an evaluation of the series as a whole, th individual ariations were eliminated by using Student's t test, the method of paired comparisons In addition t two-way analysis of variance was carried out in orde to evaluate the changes occurring during the test perio nd the difference between the tw groups, those wit and those without a diabetic predisposition

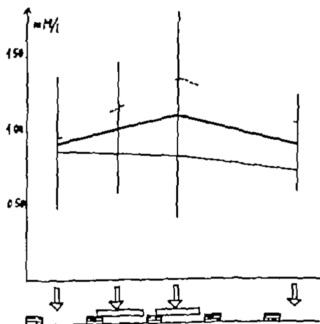


Fig 2 F sting serum triglycerides. Mea values and standard deviations of the four tests. Thick line = total series; thin line = no predisposition to diabetes mellitus, dotted line = predisposition to diabetes mellitus.

RESULTS

The fasting values for the serum triglycerides of the four sets of tests are given in Figure as mean values and the standard deviation for the series as a whole The statistical analysis of

Many studies of the serum triglycerides during the administration of oral contraceptives of the combined type have been published (4 7 12, 13 14 22, 29 30, 31). The majority of these studies have shown that the serum triglyceride concentrations were higher during hormonal administration but a single report has demonstrated unchanged concentrations (7). The variation has been attributed to the different content of hormonal derivatives in the contraceptives used (6, 7 27).

The importance of the existence in the test subjects of a predisposing factor affecting the concentration of serum triglycerides during the administration of hormonal contraceptives has been evaluated in several studies. A predisposition to diabetes mellitus has been one of the factors predisposing to changes in serum glyceride concentrations that has been given particular attention (24 29 31). In none of these studies was relationship found between a predisposition to diabetes and changes in the serum glyceride concentrations. This is in contradistinction to the observations in the present study

Metabolic changes during the administration of the same oral contraceptive as used in the present study were previously investigated by Jung (16). In this study significant increases were found in the fasting values of serum triglycerides. Fasting values of serum free fatty acids and serum insulin, response to i v glucose tolerance tests for free fatty acids and insulin were unchanged during the second cycle of hormonal administration However direct compare of this two studies is not possible. In Jungs study tests were performed later in the cycle (20–22nd day) than in the present study (14–15th day). Furthermore Jungs material does not include subjects with a hereditary predisposition to diabetes, but it does not appear to have been evaluated from the obstetric history That the latter can be considered of importance can be seen from the fact that among the 18 test subjects of Jung only 8 developed significantly greater insulin response during hormonal administration in contrast to the whole material N special analyses of this group of 8 was made in regard to the other parameters studied. In particular its fasting triglycerides are not specified, but for the whole series of 18 there is a considerable spread of these values during hormonal administration.

The mode of action by which oral contraceptives increase the concentration of serum triglycerides is not elucidated, but has been the subject of a number of studies which have pointed to various possibilities (6, 8, 10). These possibilities can be summarised as follows: 1) an hepato-toxic effect (12), 2) changed lipo-protein synthesis in the liver (10, 31), 3) increased triglyceride synthesis in the liver as a result of increased lipogenesis from carbohydrates, stimulated by insulin (13), 4) increased peripheral catabolism of triglycerides as a result of increased tissue lipase activity caused by reduced peripheral sensitivity to insulin, thereby producing increased release of free fatty acids to the circulation (20, 26). This stimulates increased synthesis of triglycerides from free fatty acids in the liver (20), 5) reduced triglyceride release from the blood owing to reduced lipoprotein lipase activity in fatty tissue (13 14), 6) increased blood level of growth hormone (25 28).

It is difficult to evaluate these various possible modes of action, several of the above suggest a connection between an effect on carbohydrate and lipid metabolism (23)

The results of the study presented here do not permit of a more detailed evaluation of the mode of action which underlies the effect on carbohydrate and lipid metabolism of the oral combined contraceptive used. In a previously published investigation of the fasting blood sugar and i.v glucose tolerance (19) carried out at the same time as the present study and on the same patients it was demonstrated that fasting blood sugar was unchanged during the administration of the hormonal contraceptive, without any difference with regard to predisposition to diabetes. Increased glucose assimulation was observed in the second cycle of hormonal administration and after the discontinuation of the hormone. It was also found that the change was independent of a predisposition to diabetes. But throughout the period of study there was a constant difference with regard to predisposition to diabetes, in such a manner that those predisposed to diabetes had a slower assimulation than those not so predisposed.

By comparing the results of these studies with studies of the physiological regulation of carbohydrate and lipid metabolism, and the changes that occur during diabetes mellitus, as described

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significantly higher response values after glucose injection. These higher responses were observed 30 min after injection in the first cycle of administration ($p < 0.001$) and 10 min after injection in the second cycle of administration ($p < 0.05$). The variance analysis demonstrated a significant difference between those predisposed and those not predisposed to diabetes in the 60 min values ($p < 0.025$) but not in the 10 and 30 min values.

DISCUSSION

Changes in the serum lipid concentrations have been found during studies on women taking oral contraceptives of the combined type (6, 10). The most constant and pronounced changes were increased concentrations of serum triglycerides (4, 13, 29, 30). However, changes in the concentration of serum cholesterol and phospholipid have also been described (7, 30).

It appears that it is mainly the oestrogen content of the contraceptive that causes the changes in lipid metabolism, particularly the increased triglyceride concentration (10, 11, 29), while the gestagens have only slight effect on this parameter.

It is presumed that with simultaneous administration of oestrogen and gestagen there is an interaction between the effects of the two components with mutual modification (6, 7, 27, 29). It also appears that there is a difference in the effect on the lipid metabolism of the various derivatives of the two components (7, 27).

The effect which the administration of an oral contraceptive will have on lipid metabolism is thus dependent both on the type of derivative of oestrogen and gestagen and on the combination of these derivatives (6, 27). An additional factor of importance can be the individual differences between the treated women. Individuals with a predisposition to the development of hyperlipidaemia can be presumed to be particularly exposed to the development of such a condition during the administration of an oral combined contraceptive (6, 14).

In the present study the parameters of lipid metabolism of the test subjects were determined not only during but also prior to and after the administration of an oral combined contraceptive, so that each individual could function as her own control. The material is divided into two groups, those predisposed to diabetes and those not so predisposed. A fixed time in relation to the menstrual cycle was used for testing. The same hormonal drug was used throughout, similarly the dosage and the duration of administration were the same. The object of planning the study in this manner was to ensure that it would be possible to evaluate by statistical analysis the effects of the oral combined contraceptive on the parameters of lipid metabolism studied here during short term administration and to determine whether predisposition to diabetes has any influence on these results. On the other hand, the planning does not permit the study to be used for evaluation of the general effects of all contraceptives on lipid metabolism or of the effects following prolonged administration.

The study showed that the fasting serum concentration of triglycerides increased during the administration of the oral contraceptive in those predisposed to diabetes, while those not so predisposed showed unchanged values throughout the period of observation. The fasting values for the free fatty acids were, for the series as a whole, lower in the second cycle of administration. There appears to be a tendency for the values of those not predisposed to diabetes to show a greater decrease during hormone administration than those predisposed to the disease, but a significant difference could not be demonstrated by variance analysis. The free fatty acids during glucose tolerance tests showed, for the series as a whole, no significant changes during hormonal administration. There appears to be, similar to the fasting values, a tendency to a lower response during hormonal administration in those not predisposed to diabetes, while this was not so for those predisposed to diabetes. However, variance analysis did not reveal any significant difference.

The serum insulin fasting values showed no changes during hormonal administration and no difference with regard to predisposition to diabetes. On the other hand the insulin response to glucose injection, for the series as a whole, was higher during hormonal administration. The greater response during hormonal administration was found both for those predisposed to and those not predisposed to diabetes, with a slightly greater response in the former group.

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significantly higher response values after glucose injection. These higher responses were observed 30 min after injection in the first cycle of administration ($p < 0.001$) and 10 min after injection in the second cycle of administration ($p < 0.05$). The variance analysis demonstrated a significant difference between those predisposed and those not predisposed to diabetes in the 60 min values ($p < 0.025$) but not in the 10 and 30 min values.

DISCUSSION

Changes in the serum lipid concentrations have been found during studies on women taking oral contraceptives of the combined type (6 10). The most constant and pronounced changes were increased concentrations of serum triglycerides (4 13 29 30). However changes in the concentration of serum cholesterol and phospholipid have also been described (7 30).

It appears that it is mainly the oestrogen content of the contraceptive that causes the changes in lipid metabolism, particularly the increased triglyceride concentration (10 11 29) while the gestagens have only slight effect on this parameter.

It is presumed that with simultaneous administration of oestrogen and gestagen there is an interaction between the effects of the two components, with mutual modification (6 7 27 29). It also appears that there is a difference in the effect on the lipid metabolism of the various derivatives of the two components (7 27).

The effect which the administration of an oral contraceptive will have on lipid metabolism is thus dependent both on the type of derivative of oestrogen and gestagen and on the combination of these derivatives (6 27). An additional factor of importance can be the individual differences between the treated women. Individuals with a predisposition to the development of hyperlipidaemia can be presumed to be particularly exposed to the development of such a condition during the administration of an oral combined contraceptive (6 14).

In the present study the parameters of lipid metabolism of the test subjects were determined not only during but also prior to and after the administration of an oral combined contraceptive so that each individual could function as her own control. The material is divided into two groups, those predisposed to diabetes and those not so predisposed. A fixed time in relation to the menstrual cycle was used for testing. The same hormonal drug was used throughout; similarly the dosage and the duration of administration were the same. The object of planning the study in this manner was to ensure that it would be possible to evaluate by statistical analysis the effects of the oral combined contraceptive on the parameters of lipid metabolism studied here during short term administration and to determine whether predisposition to diabetes has any influence on these results. On the other hand the planning does not permit the study to be used for evaluation of the general effects of all contraceptives on lipid metabolism or of the effects following prolonged administration.

The study showed that the fasting serum concentration of triglycerides increased during the administration of the oral contraceptive in those predisposed to diabetes, while those not so predisposed showed unchanged values throughout the period of observation. The fasting values for the free fatty acids were, for the series as a whole, lower in the second cycle of administration. There appears to be a tendency for the values of those not predisposed to diabetes to show a greater decrease during hormone administration than those predisposed to the disease but a significant difference could not be demonstrated by variance analysis. The free fatty acids during glucose tolerance tests showed, for the series as a whole, no significant changes during hormonal administration. There appears to be, similar to the fasting values, a tendency to a lower response during hormonal administration in those not predisposed to diabetes, while this was not so for those predisposed to diabetes. However variance analysis did not reveal any significant difference.

The serum insulin fasting values showed no changes during hormonal administration and no difference with regard to predisposition to diabetes. On the other hand the insulin response to glucose injection, for the series as a whole, was higher during hormonal administration. The greater response during hormonal administration was found both for those predisposed to and those not predisposed to diabetes, with a slightly greater response in the former gro

on the mechanism of hypertriglyceridaemia induced by oral contraceptives. *In* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A. Salhanick, D. M. Kipnis & R. L. Van de Wiele), pp. 212–41. Plenum Press, New York and London, 1969.
15. Jenkins, D. J. A.: Modern concepts of free fatty acid and blood glucose homeostasis in diseases involving altered lipid metabolism. Lancet *II* 341–344, 1967.
16. Jung, T.: Kohlenhydrat- und Fettstoffwechsel-Veränderungen während der Behandlung mit einem Ovulationshemmer. (Thesis.) Göttingen, 1969.
17. Kannel, W. B., Dawber, T. R., Friedman, G. D., Glennon, W. E. & McNamara, P. N.: Risk factors in coronary heart disease. Ann Intern Med *61* 888–899, 1964.
18. Munkner, C.: Blodets indhold af non-esterificerede fedtsyrer, triglycerid og endogen lipoproteinlipase hos nondiabetikere og diabetikere. (Thesis.) Århus, 1970.
19. Nielsen, F. Hasting: Intravenous glucose tolerance, insulin response, fasting blood glucose and serum insulin during short-term administration of an oral combined contraceptive. Acta Obstet Gynec Scand *51* 319–324, 1972.
20. Randle, P. J., Garland, P. B., Hales, C. N. & Newsholme, E. A.: The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet *I* 785–789, 1963.
21. Reaven, G. M., Lerner, R. L., Stern, M. P. & Farquhar, J. W.: Role of insulin in endogenous hypertriglyceridaemia. J Clin Invest *46*, 1756–1767, 1967.
22. Seitz, P., Haecha, H. H., Rebensburg, W. & Voigt, K. D.: Systematic investigations on the influence of contraceptive on some biochemical parameters of fat and carbohydrate metabolism. Acta Endocr *62* 181–192, 1969.
23. Spellacy, W. N.: A review of carbohydrate metabolism and the oral contraceptives. Am J Obstet Gynec *104* 448–460, 1969.
24. Spellacy, W. N., Buhi, W. C., Spellacy, C. E., Moses, L. E. & Goldzieher, J. W.: Glucose, insulin and growth hormone studies in long-term users of oral contraceptives. Am J Obstet Gynec *106* 173–182, 1970.
25. Spellacy, W. N., Carlson, K. L. & Schade, S. L.: Human growth hormone levels in normal subjects receiving an oral contraceptive. JAMA *202*, 451–454, 1967.
26. Sutherland, E. W., Robison, G. A. & Butcher, R. W.: Some aspects of the biological role of adenosine 3',5'-monophosphate (cyclic AMP). Circulation *37* 279–306, 1968.
27. Svanborg, A.: Possible relationship between plasma lipids and hormonal steroids. *I*: Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A. Salhanick, D. M. Kipnis & R. L. Van de Wiele), pp. 24? 246. Plenum Press, New York and London, 1969.
28. Wynn, V. & Doar, J. W. H.: Some effects of oral contraceptives on carbohydrate metabolism. Lancet *II* 715–720, 1966.
29. Wynn, V. & Doar, J. W. H.: Fasting serum triglyceride and cholesterol levels during oral contraceptive therapy. *In* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A. Salhanick, D. M. Kipnis & R. L. Van de Wiele), pp. 219–231. Plenum Press, New York and London, 1969.
30. Wynn, V., Doar, J. W. H. & Mills, G. L.: Some effects of oral contraceptives on serum-lipid and lipoprotein levels. Lancet *I* 720–723, 1966.
31. Wynn, V., Doar, J. W. H., Mills, G. L. & Stokes, T.: Fasting serum triglyceride, cholesterol and lipoprotein levels during oral-contraceptive therapy. Lancet *II* 756–760, 1969.

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by among others Randle (20) Reaven (21), Jenkins (15) and supported by the clinical works of Munkner (18) certain modes of action can be assumed. Furthermore this is supported by the results of studies on the metabolic changes occurring during hormonal treatment referred to previously

An increased insulin response during hormonal administration was demonstrated, presumably caused by an increased β-cell response to a higher blood sugar level in the pancreas. This has been demonstrated in earlier studies (5) as being caused by certain oestrogen and gestagen derivatives. This increased insulin response explains the raised glucose assimilation during hormonal administration It could also explain the tendency found to lower values of the serum free fatty acids in those not predisposed to diabetes, by the inhibiting effect of insulin on the lipolysis in the fatty tissue (20). The fact that there was not the same tendency to lower serum free fatty acids during hormonal administration in those predisposed to diabetes can result from the fact that they have developed a relative peripheral insulin resistance (5) by which the increased inhibition of lipolysis by the serum insulin is reduced.

There are various possible modes of action behind the increase in serum triglycerides that occurs during hormonal administration to those predisposed to diabetes 1) the greater insulin response in those predisposed to diabetes causes an increased triglyceride synthesis in the liver as a result of increased lipogenesis from carbohydrate (21), 2) increased peripheral lipolysis in those predisposed to diabetes gives a higher serum concentration of free fatty acids and these increase triglyceride synthesis in the liver (20)

As previously mentioned the present study can not be expected to give definite information as to the risk with prolonged use of oral combined contraceptives. However the results would suggest that individuals predisposed to diabetes when taking the contraceptive used in this study can run the risk of developing an increase in the serum concentration of triglycerides and with this increase the risk of developing coronary artery disease ( ).

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REFERENCES

1 Adlercreutz H Kerstell, J & Svanborg, A., Simultaneous estimation of plasma cholesterol, total and individual phospholipids, triglycerides, free fatty acids and cortisol and urinary estrogens, total 17-ketosteroids, individual 11-desoxy 17-ketosteroids, total 17-ketogenic steroids and pregnandiol during the menstrual cycle and in early pregnancy Ann Med Exp Biol Fenn 45 285–29.., 1967

2 Albrink, M J Triglycerides, lipoproteins and coronary artery disease Arch Intern Med 109 345-359 1962.

3 Andrews, W C. Oral contraception. A review of reported physiological and pathological effects. Obst Gynec Survey 26. 477–499 1971

4 Aurell, M Cramer K. & Rybo G Serum lipids and lipoproteins during long-term administration of an oral contraceptive Lancet I 791 293 1966.

5 Beck, P Effects of gonadal hormones and contraceptive steroids on glucose and insulin metabolism I Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A Salhanick, D M. Kipnis & R L. Vande Wiele), p. 97 125 Plenum Press, New York and London 1969

6. Bierman, E. L., Oral contracepti es, lipoproteins and lipid transport. I Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H A Salhanick, D M Kipnis & R L Van de Wiele), pp. 207-218 Plenum Press, New York nd London, 1969

7 Brody S., Kerstell J Nilsson L. & S anborg, A Th effects of some ov lation inhibitors on the different plasma lipid fractions. Acta Med Scand 183 1 7 1968.

8 Editorial Effects of oral contraceptives on serum lipids and lipoproteins. Lancet II .56-.57 1969

9 Editorial Metabolic effect of oral contracepti es Lancet II 783–784 1969

10 Furman, R H Gonadal steroid effects on serum lipids. I Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H A Salhanick, D M Kipnis & R L Van de Wiele) pp. 47 64 Plenum Press, New York and London, 1969

11 Furman, R H Ala povic P & How rd, R P Effects of androgens nd estrogens on serum lipids and the composition and concentration of serum lipoproteins in normo-lipemic nd hyperlipidemic states. Progr Biochem Pharma 15– 49 1967

1.. Gershberg, H H ke M & J ler Z Hypertriglyceridaemia during treatment with estrogen nd oral contracepti es. Obst Gynec 31 186–189 1968

13 Hazzard W R Spiger M J Bagdade J D & Bierman, E. L.: Studies on the mecha ism of incre sed plasma triglyceride le b induced by or l contraceptives. New Eng J Med 280 471–474 1969

14 Hazzard, W R Spiger M J & Bierman E L Studies

on the mechanism of hypertriglyceridaemia induced by oral contraceptives. *In* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A. Salhanick, D. M. Kipnis & R. L. Van de Wiele), pp 232–241. Plenum Press, New York and London, 1969
15 Jenkins, D. J. A. Modern concepts of free fatty acid and blood glucose homeostasis in diseases involving altered lipid metabolism. Lancet *II* 341–344 1967
16 Jung, T. Kohlenhydrat und Fettstoff echsel-Veränderungen während der Behandlung mit einem Ovulationshemmer (Thesis.) Göttingen, 1969
17 Kannel, W. B., Dawber T. R., Friedman, G. D. Glennon, W. E. & McNamara, P. N. Risk factors in coronary heart disease. Ann Intern Med *61* 888–899 1964
18 Memkner C. Blodets indhold af non-esterificerede fedtsyrer triglycerid og endogen lipoproteinlipase hos nondiabetikere og diabetikere (Thesis.) Århus, 1970.
19 Nielsen, F. Hamling: Intravenous glucose tolerance, insulin response, fasting blood glucose and serum insulin during short-term administration of an oral combined contraceptive. Acta Obstet Gynec Scand *51* 319–324, 1972.
20 Randle, P. J. Garland, P. B. Hales, C. N. & Newsholme, E. A. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet *I* 785–789 1963
21 Reaven, G. M. Lerner R. L. Stern, M. P. & Farquhar J. W. Role of insulin in endogenous hypertriglyceridaemia J Clin Invest *46* 1756–1767 1967
22 Seng, P. Hesche, H. H. Rebensburg, W. & Voigt, K. D. Systematic investigations on the influence of contraceptive on some biochemical parameters of fat and carbohydrate metabolism. Acta Endocr 62 181–192, 1969
23 Spellacy W. N. A review of carbohydrate metabolism and the oral contraceptives. Am J Obstet Gynec *104* 448–460, 1969
24 Spellacy W. N. Buhl, W. C. Spellacy C. E. Moses, L. E. & Goldzieher J. W. Glucose, insulin and growth hormone studies in long-term users of oral contraceptives. Am J Obstet Gynec *106* 173–182, 1970
25 Spellacy W. N. Carlson, K. L. & Schade S. L. Human growth hormone levels in normal subjects receiving an oral contraceptive. JAMA *202* 451–454 1967
26. Sutherland, E. W. Robison, G. A. & Butcher R. W. Some aspects of the biological role of adenosin3',5' monophosphate (cyclic AMP). Circulation *37* 279–306, 1968.
27 Svanborg, A. Possible relationship between plasma lipids and hormonal steroids. *I* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A. Salhanick, D. M. Kipnis & R. L. Van de Wiele), pp. 242–246. Plenum Press, New York and London, 1969
28. Wynn, V. & Doar J. W. H. Some effects of oral contraceptives on carbohydrate metabolism. Lancet *II* 715–720 1966.
29 Wynn, V & Doar J. W. H. Fasting serum triglyceride and cholesterol levels during oral contraceptive therapy *I* Metabolic Effects of Gonadal Hormones and Contraceptive Steroids (ed. H. A. Salhanick, D. M. Kipnis & R. L. Van de Wiele), pp. 219–231 Plenum Press, New York and London, 1969
30 Wynn, V. Doar J. W. H. & Mills, G. L. Some effects of oral contraceptives on serum-lipid and lipoprotein levels. Lancet *I* 720–723 1966.
31 Wynn, V. Doar J. W. H., Mills, G. L. & Stokes, T. Fasting serum triglyceride, cholesterol and lipoprotein levels during oral-contraceptive therapy. Lancet *II* 756–760 1969

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ACTION OF CLOMIPHENE CITRATE ON RAT TESTICULAR STEROID BIOSYNTHESIS IN VITRO

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Abstract. The side chain cleavage of cholesterol and of pregnenolone in rat testis preparations is stimulated in vitro by 0.5–1.0 10^{-4} M clomiphene citrate. The mechanism is thought to be analogous with that demonstrated by Hagerman and co-workers (1966) for the stimulation of placental ring A aromatization. The results seem to give additional support to the possibility of direct action of the drug on the steroidogenic organs in vivo.

The mode of action of clomiphene citrate upon the human reproductive system is still not quite clarified. The drug seems to act via the hypothalamic-pituitary system, promoting an increased secretion of LH (18). However results from in vitro studies and animal experiments as well as from some clinical investigations indicate the possibility of an additional direct action upon the steroidogenic tissues (see Table I).

The aromatization of ring A in steroidogenic tissues and the *N*-demethylation of *p*-chloro-*N* methylaniline in liver (reactions 1 and 2 in Table I) both require molecular oxygen and NADPH. Clomiphene citrate will stimulate these reactions by increasing the availability of NADPH due to non-competitive inhibition of the NADPH–Cytochrome *c*-oxidoreductase. The side chain cleavage of cholesterol yielding C_{21} steroids and of C_{21} steroids yielding C_{19} steroids (androgens and androgen precursors) also requires molecular oxygen and NADPH. From this, one might anticipate a stimulatory effect of clomiphene citrate upon these transformations. This suggestion is further supported by the fact that administration of clomiphene citrate to male patients results in increased plasma and urinary levels of C steroids (2, 6 12, 16). In most of these cases the increased C_{19} steroid levels were accompanied by a simultaneous rise in plasma or urinary LH. However Mellinger & Thompson observed a marked increase of urinary testosterone glucosiduronate in patients with elevated basal gonadotropin levels (16). This might indicat direct action of the drug upon the testicular steroid biosynthesis. Cathro and co-workers found elevated plasma levels of 4-androsten-3 17-dione and dehydroepiandrosterone, but *not* of testosterone after administration of clomiphene citrate to prepubertal boys. They pointed out the possibility of direct stimulation of the adrenal C_{19} steroid biosynthesis (6).

In order to test the possibility of a direct stimulation by clomiphene citrate of the testicular bio-

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Abbreviations and trivial names

GLC Gas liquid chromatography
LH Luteinizing hormone.
NAD Nicotinamide-dinucleotide
NADH Reduced form of nicotinamide-dinucleotide
NADPH Reduced form of nicotinamide-dinucleotide phosphate
PPO 2,5-diphenyloxazole
Dimethyl-POPOP 1,4-bis (2-(4 methyl-5-5 phenyl-oxazolyl)) benzene.
TLC Thin layer chromatography
Cholesterol 3β-hydroxy-5-cholestene
Clomiphene citrate 1 (p-2-diethylaminoethoxy-phenyl) 1,2-diphenyl-2-chloroethylene dihydrogen citrate
Dehydroepiandrosterone: 3β hydroxy-5-androsten-17 one
5 pregnenolone: 3β-hydroxy 5-pregnene-20-one
17 -hydroxypregnenolone: 3β, 17α-dihydroxy-5-pregnene-3-one
Progesterone: 4-pregnene-3,20-dione.
17 -hydroxyprogesterone. 17α-hydroxy-4-pregnene-3 20-dione.
Testosterone: 17β-hydroxy-4-androsten-17-one
Testosterone acetate: 17β-acetoxy-4-androsten-17-one.

Table I. *Summary of in vitro and animal studies concerning the effect of clomiphene citrate upon steroid biosynthesis and metabolism*

Reaction (system) studied	Tissue	Effect	Possible mode of action	References
1) Ring A aromatization (Dependent upon NADPH and molecular oxygen)	Human placental microsomes in vitro Perfused dog ovary	Stimulation	Increased availability of NADPH due to a non-competitive inhibition of NADPH-Cytochrome c-oxidoreductase. See 3)	1 8, 21
2) *N*-demethylation of *p*-chloro-*N*-methylaniline (Dependent upon NADPH and molecular oxygen)	Rat liver microsomes in vitro	Stimulation	As in 1)	7
3) NADPH-Cytochrome c-oxidoreductase (req ires NADPH)	Rat liver microsomes and human placental microsomes in vitro	Inhibition		8
4) 17β-hydroxysteroid dehydrogenase (NAD-dependent)	Guinea pig liver microsomes in vitro	Inhibition		20
5) 17β-hydroxysteroid dehydrogenase (NAD-dependent)	Preparation from mold fungus *Penicillium lilaci m*	Stimulation	Allosteric	4
6) Glutamic dehydrogenase (NADH-dependent)	Glutamate dehydrogenase from bovine liver	Inhibition		17
7) De novo biosynthesis of C_{21} C_{1} and C_{1}-steroids from acetate	Human corpus luteum slices in vitro	Inhibition	I hibition probably at a stage *before the side chain clea age* of cholesterol. See 8)	9 10, 11
8) Plasma total sterol levels	Rats in vivo	Decrease	Several compounds structurally related to clomiphene citrate (ethers and esters of diethylamino ethanol) are reported to inhibit the biosynthesis of cholesterol (13 20–22)	3

synthesis of C_{21} and C_{19} steroids, rat testicular homogenates have been used as a model system. Incubations were performed with [7 ^{3}H,26-^{14}C] cholesterol and with [7-^{3}H,21-^{14}C] progesterone and the effect of clomiphene citrate upon the side chain cleavage of these substrates was studied.

MATERIAL AND METHODS

Radioactive steroids. [7 ^{3}H] cholesterol (specific acti ity 15 Ci/mmole), [26-^{14}C] cholesterol (spec ct. 0 052 Ci/mm le) [7 ^{3}H] progesterone (spec ct. 16 Ci/mmole) and [^{2}1 ^{14}C] progesterone (spec act. 0 0556 Ci/mmole) were obtained from New England N clear Corporation Boston, Mass., USA. They wer purified by TLC befor use

Non-radioactive sterod Progesterone, 20α-hydroxy 4-pregnene 3-one and 17α-hydroxypregnenolone were pur chased from Ikapharm Ltd, Ramat-Gan, Israel, and cholesterol, 4-androsten-3 17-dione 17α-hydroxyprogesterone testosterone nd testosterone cetat f om Sigma Chemi cal Co, St Louis, Mo. USA. They were checked for purity by TLC and GLC.

Clomiphen citrat A racemic mixture at cis- and trans-clomiphene dihydrogen citrate was kindly donated by Oy Star AB Tampere Finland.

Other chemicals: NADPH w obtained from Sigma Chemical Co All other chemicals were of commercial reagent quality The solvents were redistilled before use. The only buffer solution used was Krebs-Ringer phosphat buffer pH 7 4 containing 200 mg of glucose per 100 ml It was prepared in glass-redistilled water

Prepar tion f testis hom genate Male Sprague-Dawley rats, weighing 200–.30 g were killed by a sharp blow to the back of the head The testes ere quickly emoved, decapsulated nd ashed with ice-cold buffer All subsequent operations wer performed t +4°C The testes were cut into pieces nd homogenized with 1 ml of buffer per g of tissue in Turmi household homogenizer for 60 sec The crude homogenate as immediately used in the incubation periments

Incubation w th steroids and clom phen citrate The reaction mixture contained 1.0 ml of crude rat testis homogenat 0.5 mg of NADPH and clom phene citrate from 0 t 2 10^{-4}M concentration. NADPH and clomiphene citrate were dded in buffer and the fi l lume of the eaction mixture was 1.3 ml.

3 µl of 1 10^{-4} M [7 ^{3}H,-1 ^{14}C] progesterone in ethanol containing 91 400 cpm of ^{3}H and 6 900 cpm of ^{14}C or 3 µl of approx. 0.7 10^{-4} M [7 ^{3}H,26-^{14}C]

Table II. *Fractions isolated by TLC of the samples from incubations with* [7 ^{3}H,21-^{14}C] *progesterone*

^{3}H/^{14}C-ratio of the substrate progesterone = 3.398

Fraction	Approximate R_F range	UV reaction	^{3}H/^{14}C-ratio, mean and range	Classified (tentatively identified) as
P-I	0.00–0.10	Positive	3.395 (2.930–3.895)	C_{21} steroids
P-II	0.11–0.28	Negative[a]	3.635 (2.554–4.593)	C_{21} steroids
P-III	0.29–0.35	Positive	3.120 (2.898–3.783)	C_{21} steroids (17α-hydroxyprogesterone)
P-IV	0.36–0.44	Positive	12.635 (9.813–20.484)	C_{19} steroids (4-androstene-3,17-dione)
P-V	0.45–0.55	Positive	4.218 (3.550–5.296)	C_{21} steroids (*mainly progesterone*)
P-VI	0.56–0.62	Positive	9.605 (6.236–15.569)	C_{19} steroids (testosterone as acetate)
P-VII	0.63–0.70	Positive	4.181 (3.173–5.271)	C_{21} steroids (20α-hydroxy-4-pregnene-3-one as acetate)
P-VIII	0.71–1.00	Weakly positive	5.105 (3.065–7.202) (increasing with increasing amounts of clomiphene citrate)	Unresolved mixture of C_{19} and C_{21} steroids

[a] Clomiphene citrate will give a strongly UV-positive spot in this zone.

cholesterol in ethanol, containing 300 000 cpm of ^{3}H and 314 000 cpm of ^{14}C were added to the samples. The incubation took place in 10 ml (?)-necked bottles in air on a shaking water bath at 37°C. The reaction was terminated by addition of 0.1 ml of glacial acetic acid.

Analyses of the reaction mixtures. Following carrier steroids were added as solutions in ethyl acetate: With progesterone as substrate 50 μg each of progesterone, 17α-hydroxyprogesterone, 20α-hydroxy-4-pregnene-3-one, 4-androsten-3,17-dione and testosterone. With cholesterol as substrate 50 μg each of cholesterol, 5-pregnenolone, 17α-hydroxypregnenolone, progesterone and 17α-hydroxy-progesterone. The steroids were extracted with 2 × 4 ml of ethyl acetate and the ethyl acetate layer was washed with 2 ml 8% $NaHCO_3$, 2 ml 2M NaOH, ml 8% $NaCHO_3$ and ml of water. It was evaporated to dryness under reduced nitrogen pressure and the residue was dissolved in 2.1 ml of hot methanol. 0.7 ml of water was added, the mixture was cooled to room temperature and shaken with 3 ml of pentane. The pentane layer was evaporated, dissolved in ethanol and directly taken to liquid scintillation counting.

The methanolic layer was evaporated to dryness and dissolved in 100 μl of acetone. 10 μl were taken directly to liquid scintillation counting for the determination of the total ^{3}H/^{14}C ratio. 50 μl were subjected to TLC on silica gel GF_{254} as described previously (5). The samples from the progesterone incubations were acetylated with acetic anhydride–pyridine 5:1 at 60°C for 1 hour prior to TLC. The Δ 3-ketosteroids were localized in 254 nm UV light. 3β-hydroxy-Δ-steroids (coming from cholesterol) were located by simultaneous running of standards beside the samples and visualizing the standards with $SbCl_3$. The UV-positive zones and the zones corresponding to 3β-hydroxy-Δ^{5}-steroids as well as the other zones of the plate were scraped off and eluted with 2 × 1 ml of ethanol.

To 1 ml of each eluate, 15 ml of a solution of 50 mg dimethyl-POPOP and 4 g PPO in 1 litre of toluene was added. Measurement of radioactivity was performed in a Packard ... model 3375 liquid scintillation spectrometer. After background correction, the percentage of each fraction (as % of the sum of ^{3}H) was calculated. ^{3}H/^{14}C-ratios were determinated for all fractions.

Classification of the steroid metabolites. The aim of this investigation was to study the influence of clomiphene citrate upon the rat testicular side chain cleavage of progesterone and cholesterol. A definite identification of the metabolites formed is therefore not of primary interest, but it will be necessary to classify the fractions from the TLC into C_{19}, C_{21} and C_{27} steroids. This was done on basis of their ^{3}H/^{14}C-ratios and their TLC properties. 17α-hydroxy-progesterone, 4-androsten-3,17-dione and testosterone (as acetate) formed from progesterone were tentatively identified by addition of carrier steroids (20 mg of each) and recrystallization to constant specific activity.

Qualitative TLC of progesterone samples was also performed before acetylation and the ^{3}H/^{14}C-ratios of the fractions were determinated.

RESULTS

Incubations with progesterone

After TLC of the reaction mixture eight zones were isolated from the plate and subjected to liquid scintillation counting (Table II). The results from the incubations are given in Table III. The side chain cleavage of the substrate expressed in total ^{3}H/^{14}C-ratio as well as in % of C_{19} steroid metabolites is clearly increased by the addition of clomiphene citrate. Especially the formation of testosterone is enhanced, at a clomiphene citrate concentration of 2×10^{-4} M with a factor of approx. 2.7.

The pentane phases from the defatting step contained only minor amounts of radioactivity and was not processed further.

Table III *Effect of clomiphene citrate upon the side chain cleavage of* [7 ^{3}H,21 ^{14}C] *progesterone by rat testis homogenate in vitro*

The figures represent mean values from duplicate incubations

Expt no	Clomiphene citrate, M	Incubation time (minutes)	Total ^{3}H/^{14}C-ratio	Mole % steroid (expressed as % of the sum of ^{3}H)						
				P I + P II (C_{21})	P III (C_{21})	P-IV (C)	P V (C_{21})	P VI (C_{19})	P VII (C_{21})	P-VIII ($C_{19}+C_{21}$)
1	None	60	4.210	9.2	43 4	15 4	14 8	7.5	2.0	7 7
1	0.5 10^{-8}	60	4.335	8 6	39 1	15 4	14 6	12 6	3.3	6.4
1	1.0 10^{-8}	60	4.566	10.5	38.2	17 1	12.8	11.0	2.9	7.5
1	2.0 × 10^{-8}	60	5 140	9 8	30.4	17.3	9 7	19.5	4.2	9 1
2	None	60	4 771	13.2	38 0	12.4	13 1	11.8	3 2	8.3
2	2.0 10^{-8}	60	6.280	6.8	19 8	13.3	7 0	30.0	3 6	19 5
2	None	20	4 490	12.0	41 8	11.3	14 7	10 3	2.7	7.2
2	2.0 10^{-8}	20	5.300	8.3	28.2	11.5	9.8	28.2	3.6	10 4

Incubations with cholesterol

The degree of transformation of cholesterol into C_{21} (and C_{19}) steroids was very small not exceeding 2% The zones from the TLC plates were therefore classified together in two groups. C I From the front of the UV positive progesterone spot to the solvent front. This fraction contained cholesterol and other low polar metabolites. It had a ^{3}H/^{14}C-ratio of 1 250 (1.231–1.259) compared with 1 210 for the substrate cholesterol. C II From the starting line to the front of the UV-positive progesterone spot. It consisted of four zones: Progesterone + 5-pregnenolone 17α hydroxyprogesterone + 17α hydroxypregnenolone, a third more polar zone, and finally the starting line zone. The mean ^{3}H/^{14}C ratio for this group was 2.34 (1 92–3 07). Because of the very low degree of transformation no attempts were made to identify these metabolites. For convenience this group was classified as C_{21} + C_{19} steroids.

The pentane phase from the defatting step contained 89 9 (85.5 – 93 2)% of the added ^{3}H radioactivity The ^{3}H/^{14}C-ratio was the same as for the substrate cholesterol, 1 210.

The results are shown in Table IV The observed effects are small, but a clear stimulatory effect of clomiphene upon the transformation of cholesterol into compounds with higher ^{3}H/^{14}C ratio can be seen Of the four zones in group C II only those two corresponding to progesterone + 5-pregnenolone and 17α hydroxyprogesterone + 17α hydroxypregnenolone increased their percentage with increasing amounts of clomiphene citrate. Their ^{3}H/^{14}C ratios also increased from 1.503 and 1 635 to 2.410 and 3 765 respectively

DISCUSSION

From the results presented it is evident that the side chain degradations of cholesterol and of progesterone in the rat testis are stimulated by clomi-

Table IV *Effect of clomiphene citrate upon the side chain cleavage of* [7 ^{3}H,26-^{14}C] *cholesterol by rat testis homogenate in vitro*

The figures represent mea values from duplicate incubations

Clomiphene citrate M	Incubation time (minutes)	Total ^{3}H/^{14}C ratio after defatting	Mole % steroid (expressed as % of the sum of ^{3}H)		
			C I ("C_{27} steroids") % of the ^{3}H sum from the TLC plate	C II ("C_{21} + C_{19} steroids") % of the ^{3}H sum from the TLC plate	% of initially added ^{3}H
None	60	1.[illegible]87	92.8	7.2	0 82
1 10^{-8}	60	1.320	88.7	11.3	1.20
2 10^{-8}	60	1.337	85.0	15 0	1 49

phene citrate in vitro. The effects observed are of the same magnitude as that reported by Hagerman et al. (8) for the ring A-aromatization. From this it might be assumed that the stimulatory mechanism will be similar i.e. an increased availability of NADPH due to inhibition of the NADPH-cytochrome *c* oxidoreductase system.

It may be discussed whether or not this in vitro effect will have any significance for the action of the drug in vivo. As has been pointed out by Hagerman and co-workers (8), the concentrations of clomiphene citrate used in these in vitro experiments are far above those which are to be expected in vivo. However the possibility of accumulation of the drug in the steroidogenic organs must be taken into consideration. This might result in high local concentrations of clomiphene citrate in different cells or parts of the cells. Thus, Schultz and co-workers (20) found very high concentrations in the mitochondria and microsomes of the ovary and the adrenal and in the microsomes of the liver 3 hours after a single subcutaneous injection of ^{14}C-clomiphene citrate in newborn female guinea pigs. After 25 hours, the concentration in the ovary was diminished, but remained high in the liver and increased in the adrenal. It might be assumed that repeated administration of the drug by daily oral intake will lead to increased accumulation in the target organs.

The inhibition of the de novo synthesis of steroid hormones from acetate in vitro reported by Hammerstein (9 10, 11) seems at first to contradict a direct stimulatory action of the drug in vivo. However the site of this inhibition seems to be located at stage prior to the cholesterol side chain cleavage. It might be assumed, that even after the decrease of the plasma sterol content caused by clomiphene citrate (3), the cholesterol levels in the plasma and tissue stores are high enough to provide excess substrate for the steroid hormone biosynthesis.

REFERENCES

1 Aakvaag, A & Eik-Nes, K. B *In* The Gonads (ed. K. W McKerns), p. 93 Appleton-Century-Crofts, New York 1969
2 Bardin, C. W Ross, G T & Lipsett, M B.. J Clin Endocr *11* 1558, 1967
3 Blohm, T R., Stevens, V L Kariya, T & Alig, H. N., Biochem Pharmacol *19* 2231 1970
4 Carlstrom, K. & Furuhjelm, M., Acta Obstet Gynec Scand *18* suppl. *3* 35 1969
5 Carlström, K Acta Chem Scand 26. 1718 1972.
6 Cathro, D M Saez, J M. & Bertrand, J J Endocr *50* 387 1971
7 Fahim, M S., Hall, D G & Jones, T Am J Obstet Gynec *109* 358 1971
8 Hagerman, D D Smith, O W & Day C. F Acta Endocr *51* 591, 1966.
9 Hammerstein, J Acta Endocr suppl. *119* 69 1967
10. — Acta Endocr *60* 635 1969
11 — Abst. 3rd Int Congr Horm. Steroids (Hamburg 1970). Excerpta Medica International Congress Series No. 210 Abst. No. 243 1970.
12. Harkness, R. A., Bell, E. T Loraine, J A Ismail, A A A. & Morse, W I Acta Endocr *58*. 38 1968
13 Holmes, W L. & Deitalbo, N W Am J Clin Nutr *10* 310 1962.
14 — Progr Biochem Pharmacol 2 1 1967
15 Lerner L. J Harris, D N Yiacas, E., Russell, H & Michal, I Am J Clin Nutr *23* 1241 1970
16. Mellinger R. C & Thompson, R. J Fertil Steril *17* 94 1966
17 Purunen, J & Arstila, A. Nature *213* 78 1967
18 Roy S Greenblatt, R B Mahesh, V B & Jungck, E. C Fertil Steril *14* 575 1963.
19 Schulz, K. D Statzer H & Bettendorf G., Endokrinologie *55* 22, 1969
20 Schulz, K. D Hosinal, F & Bettendorf, G Acta Endocr *68* 605 1971
21 Smith, O W & Day C. F Acta Endocr *44* 519 1963
22 Steinberg, D Adv Pharm *1* 59 1962.

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LUMBAR EPIDURAL ANALGESIA IN LABOUR

I. *Acid-base Balance and Clinical Condition of Mother Fetus and Newborn Child*

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Abstract Twenty-four full-term nulliparae and their babies were studied. Twelve received lumbar epidural analgesia with bupivacaine (Marcain-adrenalin®) and twelve conventional obstetrical analgesia with pethidine (Petidin®), chlorpromazine (Hibernal®), nitrous oxide and pudendal nerve block with prilocaine (Citanest®). The acid-base balance was determined in fetal and maternal blood during labour and in neonatal blood after birth. The newborn infants were placed in incubators after birth and several clinical parameters were recorded during the first two hours. Epidural analgesia to the mothers resulted in a lower degree of metabolic acidosis than conventional obstetrical analgesia. The clinical and blood-chemical parameters recorded in this study indicated no harmful effects on the newborn infants of set epidural analgesia to the mothers.

Epidural analgesia has been used for many years in obstetrics, but there are few studies referring to the fetus during labour and at delivery (4 9 11 15 21) and no reports have been published so far of serial observations on the child during labour at birth and in the immediate postnatal period.

This paper presents the results of a prospective study of the effects of epidural analgesia on mother and child during labour and delivery and on the newborn infant during postnatal adaptation to extra-uterine life.

MATERIAL AND METHODS

Patients

Twenty-eight healthy nulliparous women established in labour at 37–41 weeks gestation, calculated from the date of the last menstrual period, were selected. Only women aged 18–35 years and with no medical or obstetrical abnormality were included. They were randomly allotted to two groups, one group received lumbar epidural analgesia (epidural group) and the other conventional obstetric analgesia (control group).

Before analgesia was given the obstetrician evaluated the biparietal diameter and checked that the fetal head was fixed in the pelvic inlet. The fetal heart rate was recorded by direct auscultation every 15 min throughout labour. In the epidural group the fetal heart rate was also recorded every 5 min for a period of 20 min after every injection of bupivacaine. The uterine activity and the degree of bladder distension were checked regularly. Every hour and also prior to each fetal blood sample, the position of the head in the birth canal, its rotation and the degree of cervical dilatation was determined.

The duration of labour calculated from a cervical dilatation of 4–5 cm, was similar in both groups and lasted about 4 / hours in each group. The second stage lasted about 30 min in the control group and about 60 min in the epidural group. Oxytocin stimulation was used in four "epidural" mothers and one "control" mother. Six vacuum extractions were performed in the epidural group, partly due to loss of the bearing down reflex, and three in the control group, due to uterine inertia. Blood loss was registered in each delivery and was similar in both groups, being about 300 ml.

The babies were all singletons and in good condition at delivery. The mean gestational age was 40 weeks in both groups and the birth weights were within the normal range.

Four patients were removed from the study: two from the control group and two from the epidural group. These cases are presented in detail below. Clinical data on the 24 mothers, fetuses and newborns are presented in Tables I and II.

Epidural analgesia

The epidural space was entered with a Tuohy needle between L II and L III, and a catheter was inserted through the needle 4–5 cm. The outer portion of the catheter was secured on the patient's back with adhesive tape and the distal end connected to a bacterial filter (Millipore®) through which all injections were made.

Table II *Fetal and neonatal data*

Case No.	Gestational age (weeks)	Birth-weight (g)	Length (cm)	"Fetal distress"	Nuchal cord	Apgar score		Variations in	
						< 8 at 1 min	> 8 at 5 min	Blood chemistry	Postnatal course
E 1	40	3 730	53	—	—	–	+	—	—
E 2	41	3 580	53	—	—	–	+	—	—
E 3	40	3 980	56	—	—	–	+	—	—
E 4	40	3 120	49	Meconium	2	–	+	—	—
E 5	40	4 150	55	—	—	–	+	—	—
E 6	40	3 700	53	—	—	–	+	—	—
E 7	40	3 710	51	—	—	–		—	—
E 9	39	3 110	49	—	—	–	+	—	—
E 10	40	3 700	51	—	—	–	+	—	—
E 11	39	3 410	50	Bradycardia	2	–	+	High BDecf at 20′	—
E 13	41	3 050	49	Bradycardia	—	–	+	—	—
E 14	40	2 640	48	—	—	–		—	—
K 1	39	3 090	50	—	1		+	—	Hypertonic
K 4	39	3 100	49	—	—			—	—
K 5	40	3 050	49	—	1	–	+	—	—
K 6	40	3 800	52	Bradycardia	—	+	+	—	—
K 7	41	3 750	53	—	—	–		—	—
K 8	41	3 940	51	Bradycardia	Cord rupture	–		—	—
K 9	38	3 110	49	—	1	–		Low pH at 20′	—
K 10	40	3 140	50	—	—			—	—
K 11	39	2 500	48	—	—	–	+	—	—
K 12	40	4 000	51	—	—	–	+	—	—
K 13	41	3 300	53	Meconium	–	–		—	—
K 14	40	3 040	51	Meconium	—	–	+	—	—

the first fetal scalp blood sample and maternal vein sample were taken 30 min after the initial bupivacaine dose. Fetal scalp blood was collected according to the method of Saling (14) and during the first two hours after birth neonatal blood was obtained by femoral puncture (15). pH and P_{CO_2} were determined within 5 min on a Radiometer equipment consisting of the blood micro system BMS 3 and the pH Meter 71. BD_{ecf} was calculated from Siggaard-Andersen's alignment nomogram by reading the BD at a haemoglobin concentration of 5 g/100 ml (BDecf) the influence of P_{CO_2} is largely eliminated, due to the fact that the "in vivo" CO_2 titration curve almost equals the "in vitro" curve for blood samples with Hb 5 g/100 ml. The data obtained were registered on 80-column punchcards and programs constructed for statistical treatment in cooperation with Datacentralen, Karolinska institutet. Conventional statistical methods were applied. Mean values, standard deviations and correlation coefficients were calculated. In comparing the differences between mean values the Student's t-test was used.

RESULTS

Fetal and neonatal condition

Fetal bradycardia was not recorded in any case until the last 5 min before delivery. In 2 epidural and 2 control babies the fetal heart rate then dropped to 60–80 beats/min and in another epidural" and in 2 control" babies meconium-stained amniotic fluid was seen. The Apgar score at birth was normal in all cases except one control baby (K 6), who had 7 points. At 5 min the Apgar score was normal in all cases.

The Silverman-Andersen score 20 min after birth was 1 in one control and four epidural babies and 2 in two control" babies. From 40 min onwards the score was zero in all babies. The onset of the pain-stimulated cry was similar in both groups and varied from 1.5 to 2.2 sec. No differences were seen in rectal temperature, pulse rate or respiratory frequency between the two groups at 20, 40, 60 and 120 min after birth (Fig. 1). If the temperature recordings at these times were added and subjected to Student's *t*-test a probably significant difference was obtained between the mean values of the epidural and the control group ($n = 92$, difference $= 0.16°C$, $0.05 > p > 0.01$). The reflexes tested at 120 min were similar in both groups.

Table I *Maternal data*

Case no	Age	Oxytocin-stimulation	Hypotension	Duration of: Labour[a] (h)	Duration of: Second stage (min)	Analgesia: Total dose bupivacaine (mg) (no. of doses)	Analgesia: Duration of epidural block (h): 1st dose-delivery	Analgesia: Duration of epidural block (h): Last dose-delivery	Vacuum-extraction
E 1	30	+	–	10	85	50 (3)	6.5	1.5	+
E 2	21	+	–	16	75	50 (3)	7.0	1.0	+
E 3	27	–	–	14	60	40 (2)	3.0	1.5	–
E 4	24	–	–	5	40	40 (2)	3.0	1.2	–
E 5	25	+	–	14	45	50 (3)	6.2	0.3	–
E 6	25	–	–	10	90	45 (3)	5.0	1.5	+
E 7	21	–	+	7	30	53 (3)	4.0	0.8	–
E 9	27	+	–	22	75	105 (4)[b]	4.3	0.8	+
E 10	21	–	–	7	20	23 (1)	2.0	2.0	–
E 11	22	–	–	14	90	53 (3)	5.0	0.4	+
E 13	21	–	–	7	90	38 (2)	4.0	2.0	+
E 14	26	–	–	10	60	33 (2)	4.5	2.5	–
K 1	27	–	–	8	40				–
K 4	34	+	–	11	80				+
K 5	23	–	–	9	35				–
K 6	30	–	–	5	30				–
K 7	22	–	–	10	60				–
K 8	27	–	–	9	90				+
K 9	28	–	–	15	60				–
K 10	25	–	–	11	30				–
K 11	19	–	–	14	50				–
K 12	25	–	–	16	80				+
K 13	27	–	–	8	100				–
K 14	25	–	–	10	35				–

[a] Calculated from a cervical dilatation of 2 cm.
[b] 0.5% bupivacaine.

Bupivacaine 0.25% with adrenaline 1 in 200 000 (Marcaine-adrenalin®) was administered about every second hour in doses of 6–8 ml after an initial test dose of 2 ml. The mean total dose was 48.3 mg (range 32.5–105 mg) or 0.70 mg/kg bw (range 0.35–1.75 mg/kg bw) and the average number of injections given to each patient was 3. Maternal blood pressure was monitored regularly with a cuff manometer during uterine relaxation. Hypotension was defined as a fall in systolic blood pressure to a level below 100 mmHg. One patient (case E 7) had a temporary drop in blood pressure to 95/60 1½ hour before delivery but the hypotension was corrected by turning the patient to the left lateral position. No vasopressor agents were given, but as a precaution against hypotension an intravenous cannula was inserted for use in emergency. About 400 ml 5% glucose was infused during 4½ hours, so that each patient received about 1.3 mg glucose per kg bw/minute. The glucose infusion did not influence the metabolic component of the maternal acid-base balance (20).

Conventional analgesia

The control group received a single dose of meperidine (Petidin®) 100 mg and chlorpromazine (Hibernal®) 12.5 mg. After reaching a cervical dilatation of 8 cm the patients obtained 50% nitrous oxide in oxygen ad. lib. For delivery a pudendal nerve block with 10+10 ml of 1% prilocaine (Citanest®) was given to all patients in the control group.

Postnatal care

At birth the infant was placed on a pre-warmed resuscitation table and the upper airway was cleared by suction. The infant was then dried promptly and placed in an incubator with air temperature 33 to 34°C for two hours of close observation. The condition of the baby was evaluated by Apgar score at 1 min and at 5 and 10 min after birth. 20, 40, 60 and 120 minutes after birth the breathing performance of the baby was evaluated according to the Silverman-Andersen score (16), and at the same times the rectal temperature, the heart and respiratory rates and the motor activity were registered. At 120 min the following reflexes were tested: corneal reflex, anocutaneous reflex, flexion reflex, incurvation of trunk, Moro response, quality and onset of pain-stimulated cry.

Blood sampling, analyses and statistics

Blood samples were obtained simultaneously from the fetal scalp and a peripheral maternal vein at cervical dilatation of 5 and 10 cm, and from the umbilical vessels and a maternal vein at birth. In the epidural group

Table IV *The effect of maternal epidural analgesia on neonatal acid-base balance during the first two hours after birth. Comparison between the values of the control and the epidural group*

	Time period in minutes											
	20			40			60			120		
	$\bar{x}_{contr} \pm$ S.D		$\bar{x}_{epid} \pm$ S.D	$\bar{x}_{contr} \pm$ S.D		$\bar{x}_{epid} \pm$ S.D.	$\bar{x}_{contr} \pm$ S.D		$\bar{x}_{epid} \pm$ S.D	$\bar{x}_{contr} \pm$ S.D.		$\bar{x}_{epid} \pm$ S.D.
pH	7.165 ± 0.073 (11)		7.260 ± 0.082 (12)	7.243 ± 0.062 (12)	—	7.287 ± 0.037 (11)	7.295 ± 0.046 (12)		7.341 ± 0.051 (12)	7.344 ± 0.053 (12)	—	7.379 ± 0.045 (12)
P_{CO_2}	45.1 ± 10.8 (11)	—	38.1 ± 9.3 (12)	36.4 ± 6.2 (12)	—	37.6 ± 6.1 (11)	35.2 ± 6.5 (12)	—	36.0 ± 4.5 (12)	33.4 ± 5.6 (12)	—	30.5 ± 5.8 (12)
BD	11.6 ± 2.7 (11)	—	9.5 ± 2.5 (12)	10.7 ± 2.7 (12)		7.8 ± 2.5 (11)	8.7 ± 2.2 (12)		6.0 ± 2.3 (12)	6.9 ± 2.7 (12)	—	6.6 ± 2.6 (12)

$\bar{x}$ mean value. The significances are given as = $p < 0.001$ = $0.001 < p < 0.01$ = $p < 0.05$.
Figures in parentheses denote number of observations.

tabolic acidosis, and at delivery the pH of the "control" mothers were similar to that of the epidural mothers.

The fetal acid-base balance varied with the maternal acid-base balance and showed similar contrasts at Cx_5. At delivery the degree of metabolic acidosis was somewhat lower in the "epidural" babies than in the "control" babies, but did not differ as much as between their mothers.

In fetal scalp blood the pH remained about 0.1 unit below and P_{CO_2} about 15 mmHg above corresponding maternal values at Cx_5 and Cx_{10} in both groups. All mothers had a greater degree of metabolic acidosis than their fetuses. The maternal-fetal differences of BD were about 1.7 mEq/l at Cx_5 and 2.7 mEq/l at Cx_{10}. At delivery the mean differences of BD was 1.6 mEq/l in the control group and 0.9 mEq/l in the epidural group. No significant changes were found when the maternal-fetal acid-base differences between the epidural and control groups were compared at Cx_5, Cx_{10} and delivery.

The postnatal acid-base balance showed that in "epidural" babies compared to "control" babies the pH values were significantly higher at 20 and 60 min and the degree of metabolic acidosis significantly less at 40 and 60 min. The postnatal course of P_{CO_2} was similar in both groups, with an initial increase followed by a decrease towards normal levels. The pH-values were well

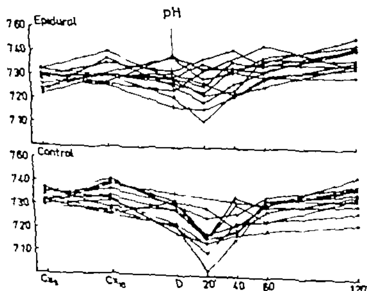


Fig. 2. pH in 12 "epidural" and 12 "control" babies during labour and after birth. Cx_5, Cx_{10} denotes cervix dilatation of 5 and 10 cm respectively. D delivery and 20' 40' 60' 120' min after birth. Neonatal blood obtained by femoral puncture as either venous (closed circles) or arterial (open circles).

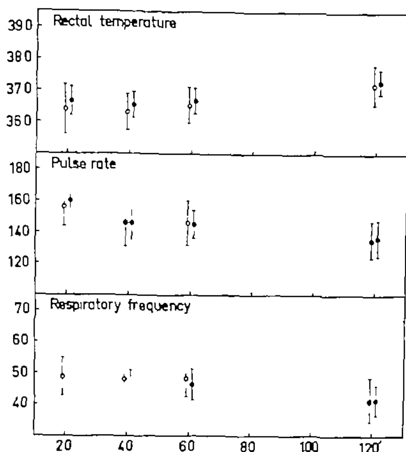


Fig 1 Rectal temperature, pulse rate and respiratory frequency in epidural" and control" babies. Mean values ±1 standard deviation recorded 20 40 60 and 120 min after birth. Closed circles denote epidural and open circles control babies.

Acid-base balance

Mean values, standard deviations and differences of mean values are presented in Tables III–IV The individual values of pH P_{CO_2} and BD_{Hb_8} in fetal and neonatal blood are given in Figs. 2–5

When the cervix was dilated 5 cm (Cx_5) the maternal pH of the control group was above that of the epidural group and correlated well with the corresponding P_{CO_2} values ($n = 12$; $r = 0\ 85$ $0\ 001 > p$). As labour proceeded this pH decreased gradually due to the increasing me

Table III *The effect of epidural analgesia on maternal and fetal acid-base balance Comparison between the values of the control and the epidural group*

	pH			Pco			BDHb8		
	$\bar{x}_{control}$ ± S.D		$\bar{x}_{epidural}$ ± S.D	$\bar{x}_{control}$ ± S D		$\bar{x}_{epidural}$ ± S D	$\bar{x}_{control}$ ± S D		$\bar{x}_{epidural}$ ± S.D
M ternal blood									
Cx_5	7 454 ± 0 066 (12)		7.374 ± 0 036 (12)	22.6 ± 4 7 (12)		29 3 ± 5 7 (12)	7 7 ± 1 8 (12)	—	7 5 ± 3 0 (12)
Cx	7 451 ± 0 076 (11)	—	7 411 ± 0.077 (11)	21 8 ± 7 0 (11)	—	6.5 + 5 7 (11)	8 4 + 4.3 (11)	—	7 5 ± 1 1 (11)
Delivery	7 362 ± 0.038 (11)	—	7.374 ± 0 035 (12)	2‿8 ± 5 (11)		28.1 ± 4 7 (12)	11 7 ‹ 2.0 (11)		8 1 + 2.‿ (1‿)
Fetal blood									
Cx_5	7.328 ± 0 025 (12)		7.278 ± 0.034 (12)	36 6 ± 4 8 (11)		44 5 ± 7 9 (11)	6 ± 2.1 (11)	—	5 3 3 8 (11)
Cx_{10}	7.330 ± 0.053 (11)	—	7.315 ± 0 044 (11)	37 3 ± 6.7 (11)	—	42.2 ± 7 5 (11)	5 7 ± 2 7 (11)	—	4 7 3 0 (11)
Delivery	7.273 ± 0.037 (11)	—	7.284 ± 0 062 (12)	34 4 ± 5 7 (11)	—	38.4 ± 7.8 (12)	10.1 ± (11)		9 + 7 (12)

$\bar{x}$ = mea value The significances re given as = p 0 001 = 0.001 < p 0 01 $p < 0\ 05$
Figures i parentheses denote number of observations.

nancy Conventional obstetric analgesia, fetal bradycardia 25 min before delivery but no fetal acidosis. Difficult extraction with vacuum extractor due to occipito-posterior position. The umbilical cord was twice around the neck and once around the shoulders. Apgar scores 4, 8 and 9. Birthweight 3 100 g, length 52 cm. Hypertonia, hyperexcitability and right-sided convulsions were observed during the first days after birth. Polygraphic examination at one week showed pathological EEG but normal echoencephalography and liquorspectrography

Case 3 (E 12). 20-year-old woman with normal pregnancy. Epidural anaesthesia with 35 mg bupivacaine. 1 oxytocin stimulation during labour. Fetal bradycardia and fetal acidosis, but no cord around the neck. Due to pronounced metabolic acidosis postnatally with pH 7.04 at 20 min, bicarbonate was injected. The infant had clinical signs of moderate dysmaturity. The clinical parameters are normal except for a somewhat low rectal temperature during the first 60 min. At 120 min the acid-base balance and the neurological examination are normal.

Case 4 (E 8). 23-year-old woman with normal and supposed full-term pregnancy. Epidural anaesthesia with 35 mg bupivacaine. 1 oxytocin stimulation because of uterine inertia. Delivery uneventful with no signs of fetal distress. Birthweight 2 200 g, length 46 cm and the clinical appearance of the baby indicated a gestational age of 36 weeks. Apgar 10 at birth. No fetal or neonatal acidosis. Except for a somewhat low rectal temperature clinical parameters were normal during the first two hours after birth.

DISCUSSION

The mothers were all considered to be clinically "normal" with "normal" pregnancies and deliveries. The total duration of labour was within the mean labour limits of Friedman in all cases. The tendency to increased duration of the second stage after epidural block made the prophylactic use of vacuum extraction necessary to exclude the deleterious effect on the fetus of a second stage exceeding one hour (5). This policy is in agreement with the electively increased number of instrumental deliveries advocated by Crawford in connection with epidural analgesia (3).

Fetal bradycardia was not recorded in any case until the last 5 min before delivery and then in two "epidural" and two "control" babies. Meconium-stained amniotic fluid was seen in one "epidural" and two "control" babies. This did not influence the Apgar scores, which were normal in all babies five minutes after birth. No abnormal variations in the postnatal course or neonatal blood chemistry were seen and all newborn infants were considered to be "normal" clinically.

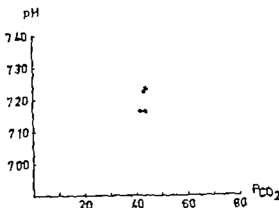


Fig. 5. Correlation of pH and P_{CO_2} in neonatal blood 20 min after birth. Epidural values = open circles; control values = closed circles (= 23 = −0.79 0.001 > p).

After birth there is an initial period of increased motor activity followed by a relatively unresponsive period in the vigorous newborn. During the first period there is tachycardia, rapid respiration and a falling body temperature and in the next period the heart and respiratory rates decline and the body temperature increases. This series of events took place in infants of both "epidural" and "control" mothers.

Newborns depressed by asphyxia or by drugs given to the mother during labour will cool more rapidly than non-medicated infants. In the present series the "epidural" babies tended to have rectal temperatures slightly higher than the "control" babies, suggesting no impairment of the normal response to cold after birth using epidural anaesthesia (2, 10).

The periods of fetal scalp blood sampling were selected according to Jacobson's classification (5) and represent periods of marked changes in fetal and maternal acid-base balance. Thus variation from the "normal" pattern could easily be detected and the results compared with Jacobson's data.

The postnatal sampling periods were selected to enable an assessment of the "normalisation" of acid-base balance to extra-uterine life. By excluding blood sampling during the first 15 min of life (17), adequate time was left for handling the baby according to the outlined schedule, the procedure of umbilical arterial catheterization could be omitted and the statistical comparison of the epidural and control groups was probably

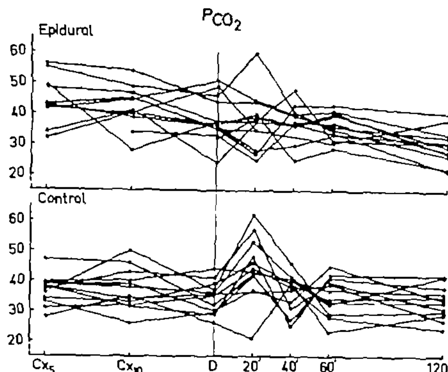


Fig 3 P_{CO_2} in 12 epidural' and 12 'control' babies during labour and after birth. P_{CO_2} in mmHg. Same legends as in Fig. 2.

correlated to corresponding P_{CO_2} values in both groups at 20 min and indicated higher P_{CO_2} values in the control" babies ($n=23$ $r=0.79$ $0.001>p$ Fig. 5).

Case descriptions

Case 1 (*K 2*). 18-year-old woman with normal pregnancy and delivery Conventional obstetric analgesia, no cord around the neck, no fetal bradycardia but increasing fetal acidosis during delivery Infant severely depressed at birth, *resuscitated by intubation, positive pressure* ventilation and injection of bicarbonate. Apgar scores 2, 5 and 9 Birthweight 2 720 g, length 51 cm. The fetal acidosis was probably caused by placental insufficiency as suggested by increased P_{CO_2} and glucose levels *in fetal blood high* lactate concentration post natally and the delivery of a small-for-gestational date infant with clinical signs of pronounced dysmaturity (19)

Case 2 (*K 3*). 22 year-old woman with normal preg-

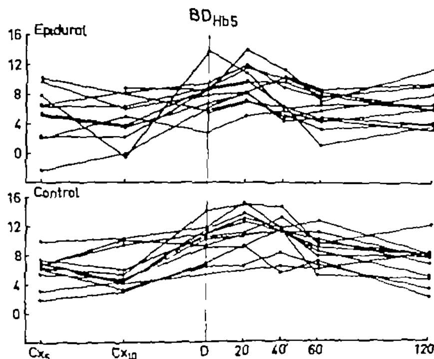


Fig 4 Metabolic acidosis in 12 'epidural and 12 'control' babies during labour and after birth. BD_{Hb5} in mEq/l Same legends as in Fig. 2.

8. — The effect of pethidine on the postnatal adjustment of respiration and acid-base balance. Acta Obstet Gynec Scand 47 27 1968.

9 Morishima, H. O Daniel, S S Finster M., Poppers, P J & James, L. S Transmission of mepivacaine hydrochloride (Carbocaine) across the human placenta Anesthesiology 27 147 1966.

10. Morishima, H. O., Mueller-Heubach, E. & Shnider S. M., Body temperature and disappearance of lidocaine in newborn puppies. Anesth Analg 50: 938, 1971.

11 Noble A. D Craft, I L., Bootes, J A. H Edwards, P A., Thomas, D J & Mills, K. L. M., Continuous lumbar epidural analgesia using bupivacaine: study of the fetus and newborn child. J Obst Gynec Brit Comm 78 559 1971

12. Reynolds, F & Taylor G Maternal and neonatal blood concentrations of bupivacaine. Anaesthesia 25, 14 1970

13 — Plasma concentrations of bupivacaine during continuous epidural analgesia in labour: the effect of adrenaline. Brit J Anaesth 43 436, 1971

14 Saling, E., Das Kind im Bereich der Geburtshilfe Georg Thieme Verlag, Stuttgart (1966).

15 Shnider S M. & Way E. L., Plasma levels of lidocaine (xylocaine) in mother and newborn following obstetrical conduction anesthesia Anesthesiology 29: 951, 1968.

16 Silverman, W & Andersen, D A controlled clinical trial of effects of water mist on obstructive respiratory signs, death rate and necropsy findings among premature infants. Pediatrics 17 1, 1956.

17 Thalme, B Electrolyte and acid-base balance in fetal and maternal blood An experimental and clinical study Acta Obstet Gynec Scand 45 suppl. 8 1967

18 Thalme, B Engström, L., Broberger U Edström, K & Kretzschmar G Infants of diabetic mothers. II. Acid-base and electrolyte balance during the first 48 hours after birth. Acta Paediat Scand 61 315 1972.

19 Thalme, B., Belfrage, P & Raabe, N., Diagnos av fetal hypoxi medelst skalpblodprov—en fallbeskrivning. Opuscula Med 17 66, 1972.

20. Thalme, B Raabe, N & Belfrage, P.: Lumbar epidural analgesia in labour II. Effects on glucose, lactate, sodium, chloride, total protein, haematocrit and haemoglobin in maternal, foetal and neonatal blood. Acta Obstet Gynec Scand. In press.

21. Zilianti, M Salazar J R., Aller J & Aguero, O.: Fetal heart rate and pH of fetal capillary blood during epidural analgesia in labor Obstet Gynec 36 881, 1970.

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facilitated due to the less marked individual varia tions in the acid-base balance 20 minutes after birth.

During conventional obstetric analgesia the initial maternal pH values were higher than during epidural analgesia. This difference in maternal blood pH was related to the probably pain stim ulated hyperventilation with a resulting lower P_{CO_2} level of the control mothers. At this early stage of labour (Cx_4) the metabolic acidosis was similar in both "epidural" and "control" mothers, but as labour progressed there was a gradual and significant increase in the metabolic acidosis of the "control" mothers. This resulted in a de creased blood pH in "control" mothers so that at the delivery the maternal pH values of the two series were in good agreement with those reported earlier (17)

The maternal and fetal acid-base balance of the control study agreed well with the normal values reported by Jacobson (5) and furthermore the concentration differences between maternal and fetal acid-base parameters were similar to that study

The neonatal pH was similar in epidural and control groups at birth, but 20 min later a high P_{CO_2} value in the control group contributed to a lower pH in these babies. This high P_{CO_2} value was probably related to the effect of meperidine on the ventilation of the newborn (6 8). The metabolic acidosis at birth and the following hour was more pronounced in "control" babies than "epidural" babies. This was probably a late effect of the over-all more pronounced metabolic acidosis of the control group before delivery

Serial blood sampling immediately after birth usually shows a combined respiratory and me tabolic acidosis, which normalises about 2 hours after birth (1 7). Due to the tendency to rather low P_{CO_2} values in both "epidural" and "control" babies in the present study such an acidosis was seen only in the control group, with its high P_{CO_2} value 20 min after birth. The low neonatal P_{CO_2} values of the present series were probably due both to the immediate, intense handling at birth, which caused the baby to cry vigorously and to the crying in connection with postnatal blood sampling.

The observation of a low degree of metabolic acidosis in epidural mothers during labour and in their babies at birth, unassociated with neonatal depression is in agreement with the studies of Hollmén (4) and Noble et al. (11).

In the present study and in the studies of Hollmén (4), Noble et al. (11) bupivacaine was used for epidural block. Bupivacaine involves less risk to the fetus than lignocaine or mepivacaine (12, 13) Furthermore, epidural block with mepivacaine (9) or lidocaine (15 21) has resulted in a low incidence of depressed infants at birth and of fetal acidosis. As these authors also pointed out there seems to be an increased fre quency of neonatal depression when large doses of local anaesthetic agents are used or in connection with falls in blood pressure. In the present study very small doses of bupivacaine were used and circulatory disturbances were minimal.

This study of epidural anaesthesia shows a less pronounced metabolic acidosis in mother and child compared with that occurring during conventional obstetric analgesia. Furthermore the clinical and acid-base parameters recorded during the early postnatal period indicated no harmful effects on the newborn infant after epidural anaesthesia to the mother

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REFERENCES

1 Berg, D & Dörrler J Das Verhalten des Saure-Basen-Haushalts am ersten Lebenstage unter besonderer Berücksichtigung der ersten Lebensminuten. Geburtsh Frauenheilk 29 980 1969
2. Burnard, E. D & Cross, K. W Rectal temperature in the newborn after birth asphyxia. Brit Med J 2 1197 1958.
3 Crawford, J S Lumbar epidural analgesia for labour Nordisk Forening for Obstetrik och Gynekologi. Seventeenth Congress, Aarhus 1972.
4 Hollmén, A. PCB och epidural anestesi. Läkartidn 68 740, 1971
5 Jacobson, L Studies on Acid-base and Electrolyte Components of Human Foetal and Maternal Blood d ring Labour Studentlitteratur Lund, 1970
6. James, L. S The effect of pain relief for labor nd delivery Anesthesiology 21 405 1960
7 Koch, G & Wendel, H. Adjustment of arterial blood gases and acid base balance in the normal inf nt dur ing the first eek of lif Biol Neonat 1 136 1968

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NORMAL BLOOD SUGAR VARIATION DURING PREGNANCY

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Abstract. Blood sugar determinations were made four times daily on non-diabetic pregnant and non-pregnant women admitted to hospital for reasons not supposed to influence carbohydrate metabolism. All blood sugar values were found to decrease during pregnancy. The fasting blood sugar decreased from 78 to 65 mg% and the daily mean value from 99 to 80 mg%. The increase in blood sugar after the main meal of the day was found to be smallest in late pregnancy. The results indicate progressive changes in carbohydrate tolerance during pregnancy. Some implications of the results on the management and outcome of diabetic pregnancy are discussed.

When managing a diabetic pregnancy therapeutic action is usually based on serial blood sugar estimations. Most authors agree that the blood sugar level should be kept within a reasonable limit. Yet there are many different opinions about which level to recommend (12).

There are many reasons to believe that the normal blood sugar level changes during pregnancy. From earlier investigations it is known that the response to glucose tolerance tests alters (1, 3, 4, 6, 10), the insulin output increases (1, 14, 15, 16) and the fasting blood sugar decreases (1, 11, 14, 15, 16).

The present investigation was made to determine how blood sugar varies during non-diabetic pregnancy. It seems reasonable to assume that the goal for the treatment of diabetic pregnancy should be to keep the mother's blood sugar level normal. The results of this investigation could form a rational basis for the control of pregnancies complicated by diabetes in the mother.

MATERIAL AND METHODS

The material consists of non-diabetic pregnant women admitted to hospital for reasons not supposed to influence glucose tolerance such as imminent abortion, suspected cervical insufficiency, incipient toxaemia, threatened premature labour etc. The non-pregnant normal cases are non-diabetic women of fertile age admitted to hospital for reasons such as salpingitis, cervical cone biopsy, minor abdominal operations etc. The number of women in each group can be seen in tables I and III.

Capillary blood samples were collected four times daily. The patients were fasting from midnight until the first sample was collected. For the rest of the day they had the regular hospital diet consisting at average of 102 g protein, 76 g fat and 246 g carbohydrate and giving approximately 2 200 Kcal/day. Blood sugar estimations were made in an auto-analyzer in the hospital's routine laboratory using a glucose oxidase method (5).

Blood samples were collected at 7 and 12 a.m. and 3 and 7 p.m. Meals were served at 7.15 and 11.15 a.m. and 4.15 and 6.30 p.m. The time relationship between meals and blood sampling can be seen in Fig. 1. The meals at 11.15 a.m. and 4.15 p.m. are the two main meals of the day.

Students t-test was used for significance testing.

RESULTS

Table I and Fig. 2 show the fasting blood sugar level in the non-pregnant women and at different times of pregnancy. The mean values decrease from 78 mg% in the nonpregnant state to 65 mg% at the end of pregnancy. The values lie close to those found by Möller (11). Table II shows that the decrease is significant between the pregnant and non-pregnant state and between early and late pregnancy.

Table III and Fig. 3 show that the daily mean values decrease from 99 mg% in the non-pregnant state to 80 mg% in late pregnancy. The differences between the mean values are significant between the pregnant and non-pregnant state and between early, mid- and late pregnancy as can be seen in Table IV.

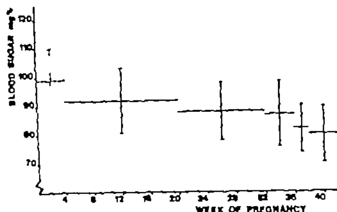


Fig. 3 Daily mean blood sugar values (M ± S.D.) at different times of pregnancy and in nonpregnant women (broken lines).

found a significant decrease in blood sugar between random samples from early and late pregnancy but the 2 1/2 hour value after oral glucose loading was unchanged during and after pregnancy. Many authors (4, 6, 10) have found a slower return to normal and a later peak value in pregnancy. The peak value is by some authors found to be higher than in the non-pregnant state (6) but other studies indicate that the peak is lower (4, 10). The differences may depend on differences in case material and methods but apparently the normal response to a glucose load in pregnancy remains to be established.

From the blood sugar values obtained at 12 a.m. in this study it can be seen that the increase in blood sugar after the 11.15 meal is greater in the non-pregnant than in the pregnant state and that the increase becomes smaller throughout pregnancy. This makes it probable that the peak blood sugar value after a physiological glucose load decreases with pregnancy even if the change in time for reaching the peak value may influence the absolute differences found here. The results also indicate that glucose tolerance changes progressively in pregnancy which has to be taken in account when evaluating glucose tolerance tests in pregnancy.

The main implication of this study concerns the management of diabetic pregnancy. Most published series (12, 13) on diabetic pregnancies have a perinatal mortality of about 10–15%. Recently two studies of great interest have been published. In the first Möller (12) showed a perinatal mortality of the same magnitude as in normal pregnancy when the mothers daily mean blood sugar level was kept below 100 mg%. The mortality in her series treated according to Pedersen (13) was 21%. The second study by Karlsson and Kjellmer (7) showed that the perinatal mortality fell from 24% in a group with a mean blood sugar above 150 mg% to 16% in an intermediate group with mean values between 100 and 150 mg% and only 3.8% in a group with levels below 100 mg%. The results of these studies lend support to the hypothesis of this study that

Table IV *Significance of difference between means of daily mean blood sugar values (p-value)*

Week of pregnancy	5–20	21–32	33–36	37–38	39–
Non pregnant	0.005	0.001	0.001	0.001	0.001
5–20		0.1	0.05	0.001	<0.001
21–32			>0.6	<0.01	0.001
33–36				0.05	0.005
37–38					0.2

Table V *Nonfasting blood sugar values during pregnancy and in non pregnant women (mg%)*

Time / Week of pregnancy	12 a.m. Mean value	S.D.	3 p.m. Mean value	S.D.	7 p.m. Mean value	S.D.	No. of patients
5–20	103	23	88	25	98	21	35
21–32	105	17	82	14	95	21	39
33–36	104	22	80	15	95	21	33
37–38	92	17	79	10	86	15	29
39–	91	14	76	13	86	15	80
Non pregnant	114	22	92	18	108	18	45

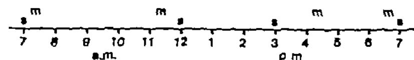


Fig 1 Time relationship between blood sampling (*s*) and meals (*m*).

Table I *Fasting blood sugar values during pregnancy and in non pregnant women* (mg%)

Week of pregnancy	Mean value (mg%)	S.D	No of patients
5–20	73	9	88
21 32	70	9	85
33–36	67	8	83
37–38	66	8	46
39–	65	9	102
Non pregna t	78	11	180

Table II *Significance of differences between mean fasting blood sugar values (p-values)*

Week of pregnancy	5–20	21 32	33–36	37–38	39–
Non pregnant	<0.001	<0.001	<0.001	<0.001	<0.001
5–20		<0.01	<0.001	<0.001	<0.001
21 32			>0.05	<0.025	<0.001
33–36				>0.5	>0.1
37 38					>0.4

The non fasting values at different times of the day are shown in Table V It can be seen that these values decrease in a way similar to the fasting and daily mean values The absolute de crease from the non-pregnant state to late preg nancy is greatest (23 1 mg%) at 12 a.m. which represents the values obtained about 45 minutes after the main meal of the day The smallest difference except from the fasting values, is be

Table III *Daily mean blood sugar values during preg nancy and in non pregnant women* (mg %)

Week of pregnancy	Mean value (mg%)	S.D	No. of patients
5–20	92	11	35
21 32	88	10	39
33–36	87	11	33
37 38	82	8	79
39–	80	10	80
Non pregnant	99	11	45

tween the 3 p.m. values (16 mg%) which re present a semi-fasting state

DISCUSSION

Glucose tolerance in pregnancy is a controversial subject. Most authors seem to agree that the insulin response to a given glucose load is en hanced and that the fasting blood sugar decreases in pregnancy The results of intravenous glucose tolerance testing are *conflicting*. *For* instance Burt et al. (2) demonstrated a relative hyper glycemic response in pregnancy whereas Spellacy et al. (14 15 16) found a lower blood sugar level throughout testing in pregnancy

Oral glucose tolerance tests during pregnancy have also given contradictory results. Lind et al. (8) found no difference between values obtained early and late in pregnancy McDonald et al. (9)

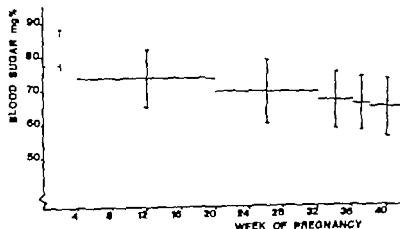


Fig 2 Fasting blood sugar values (M ± S.D) at different times of pregnancy and in nonpregnant women (broken lines)

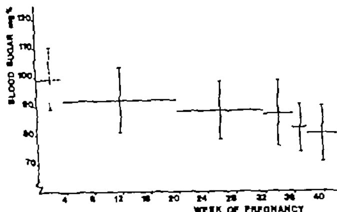


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39–	91	16	76	13	86	15	80
Non pregnant	114	22	92	18	108	18	45

the goal for the management of the pregnant diabetic should be to keep her blood sugar normal.

According to Pedersen (13) about half of the perinatal mortality in diabetic pregnancies refers to stillbirths in the majority of which the cause of death is impossible to demonstrate. About half of the neonatal deaths are due to hyaline membrane disease and a quarter to congenital malformations.

The decreased perinatal mortality is obtained mainly by the prevention of stillbirths. This decrease has occurred since a more vigorous treatment of the mothers diabetes has been instituted. This makes it probable that unexplained stillbirth may be related to the mothers blood sugar level

With a strict control of the mothers diabetes, aimed at returning her blood sugar to normal, *more patients can be allowed to deliver at term* (12) This ought to reduce the number of cases with hyaline membrane disease.

The present investigation shows that the blood sugar level is significantly decreased already in early pregnancy The study by Karlsson and Kjellmer (7) indicates that there may be a decreased malformation rate in the group with mean blood sugar values below 100 mg% at the end of pregnancy It seems probable that this group includes more mothers with well controlled diabetes in early pregnancy than the groups with higher mean blood sugar values at the end of pregnancy As a hypothesis it might be suggested that a more careful control of the mother's diabetes in the first few weeks of pregnancy could reduce the risk of congenital malformations which occur in an increased number in diabetic pregnancies.

From the results of the present investigation recommendations concerning the blood sugar levels may be suggested. The means of the daily mean values can be seen in *Table III and Fig. 2*. Upper limits for the daily mean value may be set from the mean + two standard deviations. This upper limit lies at about *115* mg% in the first half of pregnancy 108 mg% in the next 16 weeks and about 98 mg% in the last four weeks. Möller (12) reports daily mean blood sugar values very close to the values obtained in this study in her group of mothers with a mean blood sugar value below 100 mg% after the 32nd week. In her other group (mean above 100 mg%) the mean values are all above the 2 SD values of this study Yet none of her blood samples was collected as close to a meal as in the present series.

The 12 a m. values of this study represent values at least close to the peak values which can be obtained after a relatively large normal meal. From these values upper limits for single blood sugar values during the day may be set in the same way as for the daily mean values. In early pregnancy this limit lies at about 150 mg% and then successively goes down to about 125 mg% at the end of pregnancy Single blood sugar values, however must of course always be interpreted in relation to the time passed from the last meal to blood sampling

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REFERENCES

1 Bleicher S. J O'Sullivan, J B. & Frenkel, N New Engl J of Med *271* 866, 1964
2. Burt, R. L. & Leake, N. H. Obstet Gynec *33* 48, 1969
3 Burt, R. L. & Rhyne A. L. Obstet Gynec *33* 634, 1969
4 Hagen, A. Diabetes *10* 438, 1961
5 Hjelm, M & de Verdier C. H Scand J Clin Invest *15* 415 1963
6. Hohe P T Obstet Gynec *38*. 693 1971
7 Karlsson, K. & Kjellmer I Am J Obstet Gynec *11* 213, 1972.
8 Lind, T Cheyne G. A., Billewicz, W Z. & Fairweather D V I. J Obstet Gynec Brit Comm *75* 540, 1968
9 MacDonald, H N Good, W Schwartz, K. & Stone J J Obstet Gynec Brit Comm *78* 489 1971
10. Mowat, J Lindsay A. C. & McKiddie M T: J Obst t Gynec Brit Comm 77 241 1970.
11 Möller E. B Nord Med *43* 1505 1969
12 Möller E B. Studies n Diabetic Pregnancy Studentlitteratu Lund, 1970.
13 Pedersen, J The Pregnant Diabetic and Her Newborn Munksgaard, C penhagen, 1967
14 Spellacy W N & Goetz, F C New Engl J of Med *268* 988 1963
15 Spellacy W N Goetz, F C Greenberg, B Z. & Ells, J Obstet Gynec 25 86., 1965
16. — Am J Obstet Gynec 9 11 1965

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THE SIMULTANEOUS FUNCTION OF CATECHOL-O-METHYLTRANSFERASE AND MONOAMINE OXIDASE IN HUMAN PLACENTA

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Abstract Norepinephrine (NE) metabolism in 18 fresh, healthy and full-term placentas was studied. It was found that, in vitro, metabolism takes place in an oxygen atmosphere mainly through the action of monoamineoxidase (MAO). A reduction in the oxygen component pressure reduced the activity of MAO but not significantly that of catechol-O-methyltransferase (COMT). Increase in acidity also reduced MAO activity but its fall to a level as low as pH 5.4 did not significantly affect the activity of either enzyme. After the addition of $MgCl_2$ and S-adenosyl methionine a significant increase in the NMN share during 10 min incubation was noted. However these additions did not improve the decomposition of NE but reduced the proportion of DOMA. Pyrogallol failed to inhibit COMT but produced a significant reduction in the proportion of dihydroxymandelic acid (DOMA). Increase in acidity and CO_2 and the fall in O_2 content, all found in the placenta in connection with foetal asphyxia, individually reduced the decomposition of NE.

Many factors suggest that catecholamines have specific functions during pregnancy. NE,[1] which is released from sympathetic nerve endings and stimulates alpha-receptors, increases uterine contractions, whereas E, which in the main is beta-stimulant, relaxes the pregnant myometrium (2). The beta-sympathomimetics have proved to be relatively efficient in terminating premature uterine contractions (9). It is also possible that NE may play a part in the development of toxaemia of late pregnancy. It is known that the metabolism of NE and E, evaluated on the basis of the NE and E excreted in the urine (4) and of their ultimate decomposition product, VMA (18), does not seem to change either in normal or toxaemic pregnancy. However, it is only natural that increasing interest should be devoted today to the activity during pregnancy of the enzymes decomposing catecholamines, viz. MAO and COMT.

[1] Abbreviations used: E, epinephrine; NE, norepinephrine; DOMA, dihydroxymandelic acid; DHPG, dihydroxyphenyl glycol; NMN, normetanephrine; VMA, vanilmandelic acid; MHPG, methoxyhydroxyphenyl glycol; COMT, catechol-O-methyltransferase; MAO, monoamineoxidase.

The placental MAO content is high (23). Certain authors have found that the MAO activity in the placenta of a mother with toxaemia is reduced (22, 21) although apparently the activity does not decrease in a toxaemic placenta which is in good condition (15), but only when the placenta is, at the same time, degenerate (12, 5), as is often the case in toxaemia.

The placenta also contains COMT (12). COMT and MAO can even be demonstrated in the placenta at 3 months gestation. Although at this stage the MAO activity is definitely lower than at term (6), COMT activity is identical to that at full-term. Degeneration of the placenta seems to disturb the COMT less than the MAO (6). The COMT activity of a toxaemic placenta is not significantly reduced (12).

The placental catecholamines are present in the soluble fraction in the uterus, unlike the peripheral tissues in general (25). Since COMT also is located in the soluble fraction (1), it has been thought possible that COMT participates in the regulation of uterine function (8). It is therefore evident that, at least from the point of view of the fetus, and perhaps also of uterine function, it is important to know the role of MAO and COMT in the placenta. The method we used, by enabling the simultaneous observation of MAO and COMT activities, affords good possibilities for such a study because the activity of both these enzymes is required to form VMA.

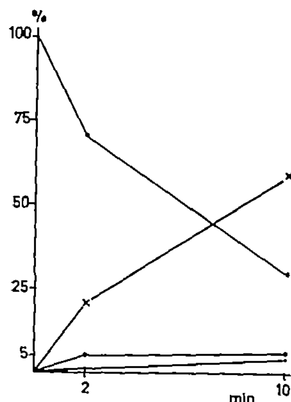


Fig 1 The metabolism of NE in placental homogenate during 2 and 10 minutes incubation in oxygen atmosphere •—• NE, ×—× DOMA o—o, NMN —— VMA.

MATERIAL

Eighteen fresh placentas obtained after uncomplicated pregnancy and delivery were examined. Macroscopically the placentas seemed to be in good condition. Identical specimens, containing all placental parts other than membrane and the membraneous vasculature were taken for study. Indispensable co-factors for the activity of pure COMT are the $-CH_3$ radical and Mg^{2+}. To ensure their presence in fresh placenta, S-adenosyl methionine (0.3 µmol/ml, $MgCl_2$ (1 µmol/ml) and ascorbic acid (0.57 mmol/ml) were used in the relevant part of the study. The MAO inhibitor used was Nialamide (0.17 mg/ml and 1.7 mg/ml), and the COMT inhibitor pyrogallol (2.65 µg/ml and 26.5 µg/ml).

METHOD

Some 3 g tissue was homogenized by Ultra-Turrax in four volumes of 0.1 M phosphate buffer. 5 ml of tissue homogenate was transferred into a 25 ml Erlenmayer flask with stopper. An oxygen atmosphere was achieved by feeding oxygen continuously through the stopper via the glass tube into the flask. After oxygenation for 2 min, 0.5 µg/g l-nor-epinephrine-7-^{3}H-HCl (The Radiochemical Centre, Amersham, specific activity 5.8 Ci/mmol) was injected into the bottle. The bottle was shaken in a machine (130 U/min) at 37°C for 2 and 10 min. The incubation was stopped by injections of 0.5 ml 4 N perchloric acid, which caused the proteins to coagulate. After centrifuging, in order to improve staining, extra NE and metabolites were added and the supernatant was neutralized with 4 N K_2CO_3 to pH 6.0. An aliquot of this solution was taken, and the NE and metabolites were separated by paper chromatography (15). Staining was carried out by means of *p*-nitroaniline. The spots were cut and burnt in a special burning device (14), in which the radioactivity of the samples was measured by liquid scintillation counting.

In order to estimate the metabolism, the percentage radioactivity of unmetabolized NE and its metabolites DOMA + DHPG, NMN and VMA + MHPG were determined.

Usually a 100% oxygen atmosphere was used in the incubation flasks, with a flow rate of 4 l/min. For assays of the effect of O_2 and CO_2 component pressures on the metabolism, the following gas mixtures were used: M I 90 mmHg O_2, 45 mmHg CO_2, and 625 mmHg N_2, and M II 45 mmHg O_2, 90 mmHg CO_2, and 625 mmHg N_2.

Table I *Effect of various gas mixtures predominant in the atmosphere on the metabolism of NE*

Mean ± S.D

Metabolites	2 min incubation: O_2 (%)	M I (%)	M II (%)	10 min incubation: O (%)	M I (%)	M II* (%)
NE	70.5 ± 4.0	76.1 ± 2.2	79.2 ± 2.7	28.5 ± 4.3	39.2 ± 2.1	42.7 ± 1.5
$p <$		0.01	0.01		0.001	0.001
DOMA + DHPG	22.4 ± 0.8	17.7 ± 2.4	15.1 ± 1.2	59.8 ± 3.8	48.8 ± 1.6	48.3 ± 2.2
$p <$		0.01	0.001		0.01	0.01
NMN	4.8 ± 0.6	4.5 ± 0.6	4.0 ± 1.1	7.0 ± 2.7	7.0 ± 2.0	5.8 ± 1.4
$p <$						
VMA + MHPG	2.3 ± 1.1	1.8 ± 0.9	1.6 ± 1.7	4.7 ± 1.1	5.0 ± 1.4	3.3 ± 0.3
$p <$						

* M I – 90 mmHg O_2, 45 mmHg CO_2, and 625 mmHg N_2. M II – 45 mmHg O_2, 90 mmHg CO_2, and 625 mmHg N_2.

Table II. Effect of the degree of acidity in the homogenate during incubation on the metabolism of NE

The metabolism of NE at pH 7.4 as the standard. Mean ± S.D.

	2 min incubation				10 min incubation			
Metabolites	pH 6.8 (%)	pH 7.4 (%)	pH 7.8 (%)	pH 8.4 (%)	pH 6.8 (%)	pH 7.4 (%)	pH 7.8 (%)	pH 8.4 (%)
NE	83.6±2.8?	73.3±4.3?	68.2±7.3?	61.7±11.0	53.8±7.3	33.9±6.0	24.9±7.4	23.7±8.1
p	0.01			0.05	0.01			
DOMA	10.2±2.7	17.2±4.8	24.8±7.6?	30.0±11.9	34.7±8.3	55.4±5.7	65.1±8.8?	64.8±10.1
p	0.05				0.01			
NMN	4.7±1.2	5.3±1.0	5.0±0.8	5.5±0.9	7.7±1.9	6.2±0.6	5.8±1.9?	6.4±2.0?
p								
VMA	1.5+0.5	2.2±1.1	2.0±0.2	2.5±0.5	3.8±2.7?	4.5±0.5	4.2±0.6	5.1±0.4
p								

RESULTS

1. Incubation in oxygen atmosphere (Fig. 1)

The metabolism of NE in 15 placental homogenates in an oxygen atmosphere was studied. During the 2 min incubation, only one-third of the NE was decomposed. One-fifth had been metabolized into DOMA, 1/20th into NMN and only 1/40 into VMA. When the incubation time was extended to 10 min, two-thirds of the NE was decomposed. For this reason it was considered that, in further studies, a reliable picture of the decomposition of NE in the placenta could be obtained by arresting the metabolism after 2 and 10 min incubation. The proportion of DOMA after 10 min incubation was almost trebled, while that of NMN remained practically unchanged. The VMA was doubled. In a good oxygen atmosphere the decomposition of NE, under these circumstances, seemed to take place mainly through the action of MAO.

2. Effect of various gas mixtures, predominant in the atmosphere, on the activity of NE decomposing enzymes (Table I)

When the oxygen content of the atmosphere was gradually reduced, it was found that the proportion of undecomposed NE increased accordingly. After 10 min incubation, under oxygen/carbon dioxide conditions approximately equal to those prevailing in the placenta towards the *end of normal pregnancy* (M I), the proportion of undecomposed NE increased highly significantly ($p < 0.01$), compared with the control test. An equally considerable increase in the proportion of NE took place after only 2 min incubation in an atmosphere which apparently corresponded to the oxygen conditions in the placenta in cases of placental insufficiency.

The weakening of metabolism seen in connection with the decrease in oxygen takes place, above all, at the expense of DOMA. After only 2 min incubation in mixture M I, its proportion had diminished significantly ($p < 0.01$) and in mixture M II, highly significantly ($p < 0.001$). A reduction in the O_2 component pressure does not significantly change the proportion of NMN. As can be expected, whenever the O_2 content of the placenta falls, the MAO, which requires oxygen for its activity, experiences difficulties more readily than COMT.

3. Effect on enzyme activity of the degree of acidity of the homogenate during incubation (Table II)

The metabolism of NE at pH 7.4 was chosen as the standard. It was found that an acidity increase to pH 6.8 inhibited enzyme activity. The amount of undecomposed NE was significantly greater ($p < 0.01$) than at the standard pH. On the other hand, acidity reduction to pH 7.8 and 8.4 caused a gradually greater though not statistically significant decrease in the NE content.

The MAO activity showed considerable sensitivity to increased acidity. After 10 min incubation at pH 6.8 the proportion of DOMA was significantly smaller than at pH 7.4 ($p < 0.01$). Reduction of acidity to pH 8.4 did not significantly change the MAO activity. These acidity changes

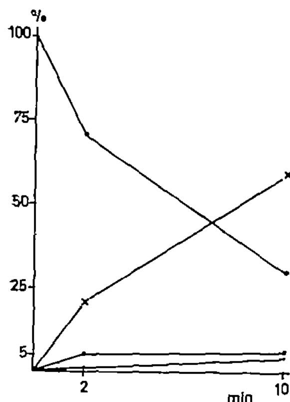


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Table I *Effect of various gas mixtures predominant in the atmosphere on the metabolism of NE*
Mean ± S D

Metabolites	2 min incubation			10 min incubation		
	O (%)	M I (%)	M II* (%)	O (%)	M I* (%)	M II* (%)
NE	70.5±2 0	76.1±2.2	79.2±2.7	28.5±2.3	39 2±2.1	42.7±1.5
p<		0.01	0.01		0.001	0 001
DOMA + DHPG	22.4±0.8	17 7±2.4	15 1±1.2	59 8±3 8	48 8±3 6	48.3±2.2
p<		0 01	0.001		0.01	0 01
NMN	4.8±0.6	4.5±0.6	4.0±1 1	7 0±2 7	7.0±2 0	5.8±1 4
p<						
VMA + MHPG	2.3±1 1	1 8±0.9	1 6±1 7	4 7±1 1	5.0±1 4	3.3±0.3
p<						

* M I – 90 mmHg O_2, 45 mmHg CO_2, and 6.5 mmHg N_2. M II 45 mmHg O_2, 90 mmHg CO_2, and 625 mmHg N_2.

Table II *Effect of the degree of acidity in the homogenate during incubation on the metabolism of NE*

The metabolism of NE at pH 7.4 as the standard. Mean ± S.D.

Metabolites	2 min incubation				10 min incubation			
	pH 6.8 (%)	pH 7.4 (%)	pH 7.8 (%)	pH 8.4 (%)	pH 6.8 (%)	pH 7.4 (%)	pH 7.8 (%)	pH 8.4 (%)
NE	83.6±2.8	75.3±4.3	68.2±7.3	61.7±11.0	51.8±7.3	33.9±6.0	24.9±7.4	23.7±8.1
$p<$	0.01			0.05	0.01			
DOMA	10.2+2.7	17.2±4.8	24.8±7.6	30.0±11.9	34.7±8.3	55.4±5.7	65.1±8.8	64.8±10.1
p	0.05				0.01			
NMN	4.7_1.2	5.3±1.0	5.0±0.8	5.7±0.9	7.7±1.9	6.2±0.6	5.8±1.9	6.4±3.0
p								
VMA	1.5_0.5	2.2±1.1	2.0±0.2	2.5±0.5	3.8±2.7	4.5±0.5	4.2±0.6	5.1±0.4
p								

RESULTS

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When the oxygen content of the atmosphere was gradually reduced, it was found that the proportion of undecomposed NE increased accordingly. After 10 min incubation, under oxygen/carbon dioxide conditions approximately equal to those prevailing in the placenta towards the end of normal pregnancy (M I), the proportion of undecomposed NE increased highly significantly ($p<$ 0.01), compared with the control test. An equally considerable increase in the proportion of NE took place after only 2 min incubation in an atmosphere which apparently corresponded to the oxygen conditions in the placenta in cases of placental insufficiency

The weakening of metabolism seen in connection with the decrease in oxygen takes place, above all, at the expense of DOMA. After only 2 min incubation in mixture M I, its proportion had diminished significantly ($p<0.01$) and in mixture M II highly significantly ($p<0.001$). A reduction in the O_2 component pressure does not significantly change the proportion of NMN. As can be expected, whenever the O_2 content of the placenta falls, the MAO which requires oxygen for its activity experiences difficulties more readily than COMT

3 Effect on enzyme activity of the degree of acidity of the homogenate during incubation (Table II)

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Table III. *Effect of agents added to promote enzyme activity on the metabolism of NE in placental homogenate*

AM = S-adenosyl methionine, Mg = $MgCl_2$, A.A. = ascorbic acid. Mean ± S.D

Metabolites	2 min incubation Control (%)	AMe (%)	Mg (%)	A.A (%)	AMeMg (%)	10 min incubation Control (%)	AMe (%)	Mg (%)	A.A. ( )	AMeMg ( )
NE	67 9±2.9	69.2±4 6	67.2±3 6	69 6±2.7	71.3±2.9	30 1±3.2	33.9±3.9	31.8±3.1	32.4±3 7	34.8±3 0
p<										
DOMA	23.9±2.5	22.3±3 0	24 1±2.4	23.4±3 1	20.6±2.3	59 6±3 0	55.8±4.2	57.2±3.1	56 4±4 0	52.8±2.7
p<										0 05
NMN	6.0±0.4	5.5±0.7	5.9±0.3	6.2±1.5	6.1±0.6	6.1±0.5	6.6±0.8	7.3±0.[illegible]	7 8±0.3	9.2±0 6
p<								0.01	0.01	0.001
VMA	2.2±0.2	2.3±0.4	2.4±0.5	2.1±0.2	2.1±0.1	4 3±0.4	3 7±0.2	3.5±0.3	3 4±0.3	3 4±0 3
p<								0 05	0.05	0 01

produced no significant alterations in the proportion of NMN and VMA.

4 *Effect of agents added to promote enzyme activity* (Table III)

The addition to the placental homogenate of the co-factors necessary for the activity of the pure COMT enzyme viz. $-CH_3$ radical and Mg^{2+} S-adenosyl methionine, ascorbic acid and magnesium, did not significantly change the proportion of NE. When S-adenosyl methionine and magnesium were added simultaneously the proportion of DOMA decreased during 10 min incubation almost significantly ($p<0\,05$) The proportion of NMN increased when magnesium ($p<0\,01$), ascorbic acid or S-adenosyl methionine, together with magnesium ($p<0.001$) were added to the homogenate. Under the influence of magnesium and ascorbic acid, the proportion of VMA also decreased slightly ($p<0\,05$) and under the influence of simultaneous S-adenosyl methionine and magnesium, distinctly ($p<0\,01$), during a 10 min incubation.

5 *Effect of MAO and COMT inhibitors on the metabolism of NE in the placenta* (Table IV)

Nialamide, added in advance to the homogenate was found to increase the proportion of undecomposed NE significantly during 10 min incubation when the dose used was 0.17 mg/ml ($p<0\,01$), and highly significantly when the dose was 1 7 mg/ml ($p<0\,001$) The DOMA fell proportionately Nialamide did not affect the amounts of NMN and VMA.

A dose of pyrogallol 2.65 μg/ml did not affect the decomposition of NE A ten fold increase in

Table IV *Effect of COMT and MAO inhibitors on the metabolism of NE in placental homogenate*

Mean ± S D N = nialamide, P = pyrogallol Mean ± S D

Metabolites	2 min incubatio Control (%)	N I[a] (%)	N II (%)	P I (%)	P II (%)	10 m[illegible] ncubation Control ( )	N I ( )	N II ( )	P I ( )	P II ( )
NE	70.9±4.8	73 6±2.9	78.3±2.9	65 6±1 9	75 6±1 4	30 5±4 8	43 9±9 4	54 7+13 7	37 0±0.3	46 3_1 5
p<			0.05				0.01	0 001		0 001
DOMA	21.5±4.5	17 7±2.6	13.6±2.4	[illegible]6.1±1 6	17 0±1 4	58 6±5 0	46 0±8.9	35.4±11.5	63 8+1 8	41 0±1.2
p<			0.05				0.05	0 001		0 001
NMN	5 4±0.9	6.7±0 2	5 9±0.7	6.0±0 5	5 8±0 4	6 5±1 7	6.1±0.5	6 6±1 4	6.7 1 6	8 4±0 4
p<		0.05								0 05
VMA	2.3±0 9	2.2±0 4	2.3±0.[illegible]	1 8±0 2	1 6±0.1	4 5±0.8	4 0±0 1	3.3±0 5	2 5±0 2	2 1_0.2
p<									0 001	0 001

N I = [illegible]ialamide 0.17 mg/ml N II = nialamide 1 7 mg ml P I = pyrogallol 2.65 μg ml P II = pyrogallol 26.5 μg/ml

concentration produced an inhibition of the NE decomposition ($p<0.001$) during the 10 min incubation. A pyrogallol concentration of 26.5 μg/ml reduced the proportion of DOMA in relation to the standard ($p<0.01$), and slightly increased that of NMN ($p<0.05$). The VMA proportion declined distinctly with an even smaller dose of pyrogallol ($p<0.001$).

DISCUSSION

The placenta contains a great deal of MAO and at least some COMT also. For this reason the placenta is well suited to serve as tissue homogenate when the simultaneous effect of these two enzymes on NE metabolism is studied.

In vitro, the NE in the brain and heart tissue of experimental animals has been found to be decomposed mainly through the action of MAO as oxidative deamination, whereas only minor O-methylation takes place through the action of COMT (7). In the circumstances of the present study the NE metabolism in the placental tissue also seemed to take place in the same way. In vivo, the NE decomposition takes place primarily by the O-methylation route (10). Our preliminary studies in the feto-placental unit indicated that NE is decomposed in the placenta, in vivo, by both COMT and MAO (20). Although the present results, therefore, are not directly comparable with results obtained under corresponding conditions in vivo, they provide additional information on the behaviour of these enzymes in a tissue little studied for this aspect, the placenta.

The placental MAO has been found to require abundant oxygen for its activity (24). The declining proportion of DOMA as the oxygen content of the tissue homogenate falls reveals clearly the dependence of MAO on oxygen. The COMT activity on the other hand, does not seem equally dependent on change in oxygen content.

The activity range of pure MAO is pH 6–10 (the optimum for tyramine is pH 7.3), and the optimum range of pure COMT is pH 7.5–8.. Under the circumstances applied in the present study MAO seemed to be sensitive also to increase in acidity since a statistically significant fall in the proportion of DOMA was recorded as pH fell from 7.4 to 6.8. COMT activity by contrast, did not deteriorate as acidity increased, which is indicated by the slight, though statistically not significant, increase in the share of NMN. As could be expected, considering the optimum ranges of the two enzymes, a fall of acidity to a level as low as pH 8.4 at least did not impair their activity in the placenta.

The study showed that fresh placental homogenate contains all the co-factors necessary for the activity of MAO and COMT. It was found, however that when $MgCl_2$ and S-adenosyl methionine were simultaneously added to the homogenate, the proportion of NMN in a 10 min incubation increased by nearly 50% compared with the standard. At the same time the proportion of DOMA fell by 7%. It is therefore possible that the amounts of Mg^{2} and $-CH_3$ contained in placental tissue are relatively small and do not suffice to maintain the full rate of COMT activity for 10 min of incubation. During an incubation of 2 min, no difference could be shown between the NE metabolism of the homogenates. It seemed that additional agents did not improve the NE decomposition. For this reason, the present authors did not find it useful to add them to the homogenate while the other factors, listed above, affecting the metabolism of NE were being studied.

It emerged clearly in the study that Nialamide had an MAO-inhibiting effect. Again it is notable that while the proportion of DOMA from NE metabolism is reduced as a result of the inhibited MAO activity the proportion of NMN remains unchanged. Consequently the COMT activity is not more marked, but the result is that the proportion of undecomposed NE increases. The reason may be that discussed above, or NMN as substrate for MAO may be more active than NE.

In the doses and under the conditions of the present study pyrogallol did not act as a COMT inhibitor whereas it did reduce the proportion of DOMA highly significantly and, contrary to expectations, slightly increased the proportion of NMN. It is evident that increasing the pyrogallol dose would not have changed the situation, since even the dose used distinctly inhibited MAO. It has been previously reported that, in vitro pyrogallol does not inhibit COMT in the heart and brain tissue of experimental animals (7, 16).

The placental MAO has been considered to have an important role in the protection of the fetus. MAO-inhibitors can kill the fetus (19), apparently due to the vasoconstrictor effect of sero-

tonin (11) It is understandable that the placenta contains MAO which has its location in cell mitochondria It is believed to be responsible for the destruction not only of serotonin but also of any excessive NE stored in the tissues (3). As a result, the MAO inhibitor iproniazide, causes the tissue content of endogenous NE to increase (17). It is also probable that COMT is not contained in the sympathetic nerve but in the adjacent cells (13)

The role of COMT in the placenta has so far not been clarified. Appreciable concentrations of NE and E transfer the placenta (23). The functions of COMT may be to prevent the passage through the placenta of an excessive amount of biologically active catecholamines noxious to the foetus.

From the clinical point of view the study was interesting because MAO activity was found to be disturbed under conditions seen in the placenta during pathological states of pregnancy such as anoxia excess of carbon dioxide, and an environment more acid than normal In vivo the differences in parameters recorded individually are not so pronounced as those used in the present study In spite of this, it is possible that even smaller differences but in a large number of parameters may if simultaneously present, produce such a high deficit in NE destruction that an amount of NE noxious to the fetus can pass through the placenta

ACKNOWLEDGEMENTS

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REFERENCES

1 Axelrod, J & Tomchick, R Activation and inhibition of adrenaline metabolism Nature (Lond) 184 2027 1959
2. Baumgarten K Die Beeinflussung der Uterusmotilität. Verlag Bruder Hollinek, Wien, 1967
3 Brodie, B B Spector S. & Shore, P A Interaction of drugs with norepinephrine in the brain. Pharmac Rev 11 548 1959
4 Castrén, O Urinary excretion of noradrenaline and adrenaline in late normal and toxemic pregnancy Acta Pharmac Toxic 20 Suppl. 2 1963
5 Castrén, O Kaartinen, N & Saarikoski, S. The metabolism of H³ noradrenaline in human placental and foetal tissue homogenates. Ann Chir Gynaec Fenn 57 405 1968
6 Castrén, O & Saarikoski, S. The effect of some different factors on the metabolism of H³-noradrenaline in placental, adnexal and foetal tissue homogenates. Acta Obstet Gynec Scand 48 Suppl. 3, 151 1969
7 Crout, J R Creveling, C. R. & Udenfriend, S Norepinephrine metabolism in rat brain and heart. J Pharmac Exp Ther 13 269 1961
8. Giles, R. E. & Miller J W. The catechol-O-methyl transferase activity and endogenous catecholamine content of various tissues in the rat and the effect of administration of U-0521 (3 4-dihydroxy -methyl propiophenone). J Pharmac Exp Ther 158 189 1967
9 Hendricks, C. H Cibils, L. A Pose S. V & Eskes, T K The pharmacologic control of excessive uterine activity with isoxsuprine Amer J Obstet Gynec 82 1064 1961
10. Hertting, G & Axelrod, J Fate of tritiated noradrenaline at the sympathetic nerve endings. Nature (Lond.) 192 172, 1961
11 Honey D P., Robson, J M & Sullivan, F M Mechanism of inhibitory action of 5-hydroxytryptamine on placental function. Amer J Obstet Gynec 99 250 1967
12. Ilsalo, E. & Castrén, O The enzymatic inactivation of noradrenaline in human placental tissue. Ann Med Exp Fenn 45 253 1967
13 Iversen, L L., Glowinski, J & Axelrod, J The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. J Pharmac Exp Ther 151 273 1966.
14 Kaartinen, N A new oxidation method for the preparation of liquid scintillation samples. Packard Techn Bull No. 18 1969
15 Klinge, E., Penttila, O & Tissari, A The content of 5-hydroxytryptophan decarboxylase and monoamine oxidase activities in normal and toxaemic pregnancies. Acta Obstet Gynec Scand 48 107 1969
16. Maitre, L Effect of long-term administration of pyrogallol on tissue catecholamine levels, monoamine oxidase and catechol-O-methyltransferase activities in the rat. Biochem Pharmac 11 1109 1962
17 Pekkarinen, A Castrén O & Ilsalo, E. Effect of iproniazide and ganglionic blocking substances on the noradrenaline content of tissues. Abstr Internat. Symposium on the catecholamines in cardiovascular pathology Burlington, p 14a 1959
18. Pekkarinen, A & Castrén O Excretion of vanillmandelic acid (VMA) in the third trimester of normal and toxaemic pregnancy and other clinical conditions. Ann Chir Gynaec Fenn 57 373 1968
19 Poulsen, E & Robson, J M Effect of phenelzine and some related compounds on pregnancy and sexual development J Endocrin 30 205 1964
20 Saarikoski, S. & Castrén, O Distribution and metabolism of H³-noradrenaline in various tissues of the human foetus in the foetoplacental unit Acta Obstet Gynec Scand 50 60, 1971
21 Sagone I & Arrotta, U Rapporti tra serotonina e monoaminoossidasi placentari nella gestosi eclamptica. Ann Ostet Ginec 88 81 1966.
22 Sandler M & Coveney J Placental monoamine-oxidase activity in toxemia of pregnancy Lancet I 1096 1962.

23. Sandler M, Ruthven, C. R. J. & Wood, C.: Metabolism of C^{14}-norepinephrine and C^{14}-epinephrine and their transmission across the human placenta. Int J Neuropharmac 3: 123, 1964
24. Thompson, R. H. S. & Tickner, A.: Observations on the monoamine oxidase activity of the placenta and uterus. Biochem J 45: 125, 1949
25. Wurtman, R. J., Axelrod, J. & Potter, L.: The disposition of catecholamines in the rat uterus and the effect of drugs and hormones. J Pharmac Exp Ther 144: 150, 1964

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Finland

tonin (11). It is understandable that the placenta contains MAO which has its location in cell mitochondria. It is believed to be responsible for the destruction not only of serotonin but also of any excessive NE stored in the tissues (3) As a result, the MAO inhibitor iproniazide, causes the tissue content of endogenous NE to increase (17) It is also probable that COMT is not contained in the sympathetic nerve but in the adjacent cells (13).

The role of COMT in the placenta has so far not been clarified Appreciable concentrations of NE and E transfer the placenta (23). The functions of COMT may be to prevent the passage through the placenta of an excessive amount of biologically active catecholamines noxious to the foetus.

From the clinical point of view the study was interesting because MAO activity was found to be disturbed under conditions seen in the placenta during pathological states of pregnancy such as anoxia, excess of carbon dioxide and an environment more acid than normal In vivo the differences in parameters recorded individually are not so pronounced as those used in the present study In spite of this, it is possible that even smaller differences but in a large number of parameters may if simultaneously present, produce such a high deficit in NE destruction that an amount of NE noxious to the fetus can pass through the placenta.

ACKNOWLEDGEMENTS

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REFERENCES

1 Axelrod, J & Tomchick, R. Activation and inhibition of adrenaline metabolism. Natur (Lond.) *184* 2027 1959

2. Baumgarten, K., Die Beeinflussung der Uterusmotilität. Verlag Brüder Hollinek, Wien, 1967

3 Brodie, B B Spector S. & Shore P A Interaction of drugs with norepinephrine in the brain. Pharmac Rev *11* 548 1959

4 Castrén, O Urinary excretion of noradrenaline and adrenaline in late normal and toxemic pregnancy Acta Pharmac Toxic *20* Suppl. 2, 1963

5 Castrén, O Kaartinen, N & Saarikoski S. The metabolism of H³-noradrenaline in human placental and foetal tissue homogenates. Ann Chir Gynaec Fenn *57* 405 1968

6. Castrén, O & Saarikoski, S. The effect of some different factors on the metabolism of H³-noradrenaline in placental, adnexal and foetal tissue homogenates. Acta Obstet Gynec Scand *48* Suppl. 3 151 1969

7 Crout, J R., Creveling, C. R & Udenfriend, S Norepinephrine metabolism in rat brain and heart. J Pharmac Exp Ther *13* 269 1961

8 Giles, R E. & Miller J W The catechol-O methyl transferase activity and endogenous catecholamine content of various tissues in the rat and the effect of administration of U-0521 (3 4 -dihydroxy-α-methyl propiophenone). J Pharmac Exp Ther *155* 189 1967

9 Hendricks, C. H., Cibils, L. A Pose S. V & Eskes, T K. *The pharmacologic control of excessive uterine* activity with isoxsuprine Amer J Obstet Gynec *8*.. 1064 1961

10 Hertting, G & Axelrod, J Fate of tritiated noradrenaline at the sympathetic nerve endings. Nature (Lond.) *19* 172, 1961

11 Honey D P Robson, J M & Sullivan, F M Mechanism of inhibitory action of 5-hydroxytryptamine on placental function. Amer J Obstet Gynec 99 250 1967

12. Iisalo E. & Castrén, O The enzymatic inactivation *of noradrenaline in human placental tissue* Ann Med Exp Fenn *45* 253 1967

13 Iversen L. L., Glowinski, J & Axelrod, J The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. J Pharmac Exp Ther *151* 273 1966.

14 Kaartinen, N A new oxidation method for the preparation of liquid scintillation samples. Packard Techn Bull No. 18 1969

15 Klinge E., Penttilä, O & Tiusari, A The content of 5-hydroxytryptophan decarboxylase and monoamine oxidase activities in normal and toxaemic pregnancies. Acta Obstet Gynec Scand *43* 107 1969

16. Mahre L Effect of long-term administration of pyrogallol on tissue catecholamine levels, monoamine oxidase and catechol-O-methyltransferase activities in the rat. Biochem Pharmac *11* 1109 1962.

17 Pekkarinen, A Castrén, O & Iisalo, E. Effect of iproniazide and ganglionic blocking substances on the noradrenaline content of tissues. Abstr Internat. Symposium on the catecholamines in cardiovascular pathology Burlington, p. 14a 1959

18 Pekkarinen, A & Castrén O Excretion of vanillmandelic acid (VMA) in the third trimester of normal and toxaemic pregnancy and other clinical conditions. Ann Chir Gynaec Fenn *57* 373 1968

19 Poulsen, E. & Robson, J M. Effect of phenelzine and some related compounds on pregnancy and sexual development. J Endocrin *30* 205 1964

20. Saarikoski, S. & Castrén, O Distribution and metabolism *of H³ noradrenaline in various tissues* of the human foetus in the foetoplacental unit Acta Obstet Gynec Scand 50 69, 1971

21 Sagone L & Arrotta, U Raporti tra serotonina e *monoaminoossidasi placentari nella gestosi eclamptica* Ann Ostet Ginec *88* 81 1966.

22. Sandler M & Coveney J Placental monoamineoxidase activity in toxemia of pregnancy Lancet *I* 1096 1962.

Acta Obstet Gynec Scand 53 49–58, 1974

CONTENT OF β_2 MICROGLOBULIN AND ALBUMIN IN HUMAN AMNIOTIC FLUID

A Study of Normal Pregnancies and Pregnancies Complicated by Haemolytic Disease

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Abstract. The content of the low-molecular weight protein β_2-microglobulin and albumin was measured by immunochemical methods in amniotic fluid from 136 patients in the 13th to the 43rd week of pregnancy. Parallel estimations of β_2-microglobulin in maternal plasma were made in the majority of patients. Some samples of cord serum and urine from the newborn were also analysed. In normal patients the levels of β_2-microglobulin followed the albumin pattern with a peak value around the 24th week and a gradual decrease during the last trimester. In contrast to other proteins β_2-microglobulin showed higher levels in amniotic fluid than in maternal plasma. During the last trimester levels in maternal plasma exhibited a significant increase. Levels in cord serum were higher than in amniotic fluid. Urine from the newborns contained at least 5 times the amount previously found in adults. The results indicate that β_2-microglobulin in amniotic fluid is largely derived from the fetus itself. Patients with pregnancies complicated by haemolytic disease showed significantly higher levels in amniotic fluid than normal patients for comparable periods but the levels showed no close correlation with the degree of fetal involvement. The ratio of β_2-microglobulin to albumin in amniotic fluid was elevated in patients with fetal haemolytic disease. The albumin content showed no significant differences between the clinical groups but tended to decrease more rapidly during the last weeks of pregnancy in patients with fetal haemolytic disease. Further studies of β_2-microglobulin might supply important information about the transfer mechanisms of amniotic fluid proteins and also permit studies of some physiological and pathological processes in the fetus.

In 1968 Berggård & Bearn (2) described a low-molecular weight protein, β_2-microglobulin (molecular weight 11 800), occurring in human urine, plasma and cerebrospinal fluid. It has also been found in human saliva and colostrum (10). Own preliminary studies indicated that amniotic fluid also contained comparatively large amounts of this protein. β_2-microglobulin is present in increased amounts in the urine from patients with impaired renal tubular function (2, 25). In healthy subjects the serum concentration varies within fairly narrow limits and slightly increases with age (11). Somewhat elevated serum levels have been reported in malignant disease and some disorders possibly connected with an abnormal immune response (12). However, high serum levels are usually due to an impaired glomerular filtration rate and in renal disease a strong correlation exists between the serum concentration of β_2-microglobulin and the clearance of inulin (34).

The real biological role of β_2-microglobulin is still largely unknown. Evidence is accumulating that lymphocytes produce it (3). The similarity between the amino-acid sequence of β_2-microglobulin and the constant portions of the immunoglobulins was first reported by Smithies & Poulik (30). Homology with the C_H3 region is especially striking and the protein has thus been suggested to represent a free immunoglobulin domain but is not considered to be a breakdown product of the immunoglobulins (24).

Previous studies of proteins in amniotic fluid have usually considered proteins of higher molecular weights. It was therefore thought to be of interest to study the low-molecular weight β_2-microglobulin parallel with estimations of albumin (molecular weight 69 000) during different phases of normal pregnancy and to compare these levels with the levels from patients with pregnancies complicated by fetal haemolytic disease.

Table I. *Content of β_2-microglobulin in amniotic fluid and maternal plasma during the second trimester of normal pregnancy*

Albumin content of amniotic fluid. Ratio of β_2-microglobulin to albumin in amniotic fluid. Means ± S.E.M. – number of samples

Week of pregnancy	β_2 micro in amniotic fluid		β_2 micro in maternal plasma		Albumin in amniotic fluid		$\frac{\beta_2 \text{ micro amn. fl.}}{\text{Albumin amn. fl.}} 10^4$	
		µg/ml		µg/ml		mg/ml		
13–14	4	4.53 ±0.95	3	1.55 ±0.23	3	1.50 ±0.45	3	4.16 ±1.14
15–16	7	7.36 ±1.12	5	1.41 ±0.17	7	2.07 ±0.40	7	3.70 ±0.25
17–18	7	9.32 ±0.72	8	1.50 ±0.19	7	2.46 ±0.1	7	3.97 ±0.46
19–20	9	9.33 ±0.65	11	1.24 ±0.09	9	3.52 ±0.39	9	2.88 ±0.32
21–22	2	12.90 —	2	1.45 —	2	3.65 —	2	3.41 —
23–24	5	12.88 ±1.16	5	1.54 ±0.19	5	3.78 ±0.50	5	3.61 ±0.50

the mean values was observed (Fig. 2). The decrease from the weeks 23–24 to the weeks 35–36 was highly significant. The further decrease to the end of pregnancy was not significant. No significant correlation existed between the amniotic fluid levels of β_2-microglobulin and the corresponding levels in maternal plasma during the second ($n = 30$) or the third trimester ($n = 35$). No significant correlation existed between the amniotic fluid levels and the levels in cord serum of infants delivered within 7 days of amniocentesis ($n = 14$).

Rh-isoimmunized patients with affected fetuses (group A + B) showed probably significantly elevated mean values in the 35th–36th and the 37th–38th weeks compared with the normal patients (Fig. 2). The differences between groups A and B were probably significant only in the 33rd–34th week. Individual values for patients with infants who did not survive fell within the same range as

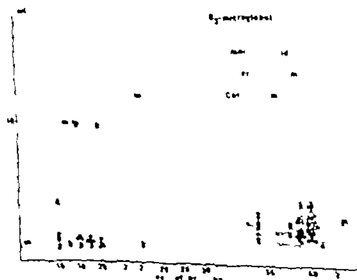


Fig. 1. Individual levels of β_2-microglobulin in normal subjects. Lines connect consecutive values from the same patient.

MATERIAL

Amniotic fluid was collected by abdominal amniocentesis from patients not in labour unless otherwise stated. Duration of pregnancy was calculated from the first day of the last menstrual period. Patients with unreliable menstrual data were not included in the study. Samples contaminated by visible amounts of blood or meconium were also excluded. Amniotic fluid samples were centrifuged and filtered within 2 hours of amniocentesis and then stored frozen at −18 C until analysis. The technique of amniocentesis was described previously (18).

Amniotic fluid was obtained from 38 patients admitted for therapeutic abortion in the 13th to the 24th week. All these patients were without signs of isoimmunization and were clinically healthy. In the later phase of pregnancy 39 samples of amniotic fluid was collected from 33 patients with clinically normal pregnancies. Most of these samples came from patients with isoimmunization in a previous pregnancy. The present pregnancy resulted in a healthy baby negative for the involved antigen and with a negative direct Coombs test on cord blood. Some samples were taken during elective caesarean section for contracted pelvis. In an additional 9 patients with normal pregnancies samples of amniotic fluid were taken vaginally during normal delivery and in these cases samples of cord blood and infant urine were taken just after delivery.

From 48 patients with Rh-isoimmunization and affected fetuses 70 samples of amniotic fluid were obtained. These samples were taken in connection with the routine management of isoimmunized pregnant patients. All these patients gave birth to infants who were Rh positive and had a positive direct Coombs test on cord blood. These patients were divided into two groups according to the degree of infant anaemia.

Group A

Infants without manifest anaemia at birth. Thirty-three samples from 25 patients. Cord blood haemoglobin concentration > 12.1 g/100 ml. Two infants in this group died postnatally because of respiratory distress.

Group B

Infants with manifest anaemia at birth. Thirty-seven samples from 23 patients. Cord blood haemoglobin < 12.1 g/100 ml. Four infants died after birth. In two death was due mainly to erythroblastosis, and in the other two postnatal complications were considered to be the main cause of death. The cord blood haemoglobin value of 12.1 g/100 ml was chosen as the limit between the groups as it may be considered to represent the lower limit of normal in this series (16).

Samples were also obtained from 8 patients with other complications of pregnancy. Four patients had hepatosis gravidarum, 2 had preeclamptic toxaemia, one had diabetes and one had polyhydramnios without fetal malformations.

Samples of maternal venous plasma were taken the same day as amniocentesis and in some cases also samples of cord serum could be collected at delivery.

METHODS

Determinations of β_2-microglobulin were performed by a radioimmunoassay (10). When tested by 15 repeated duplicate determinations of the same serum and urine this method gave a coefficient of variation of 9.4 for the serum sample and 8.-% for the urine sample. The range of measuring was -50 ng/ml, and the samples were diluted to bring the concentrations within this range. All determinations were performed in duplicate at two dilutions.

Albumin in amniotic fluid was assayed by single radial immunodiffusion (22). Standard and antiserum were obtained from Behringwerke AG, Marburg. Eighteen repeated duplicate determinations of the same serum gave a coefficient of variation of 6.8%. Serum was diluted 1 1 000 and amniotic fluid 1 30 before assay. In 26 samples of amniotic fluid parallel determinations of the albumin content were made by calculation from runs of free zone electrophoresis (17). The immunodiffusion technique gave a mean of 1.55 ± 0.21 (S.E.M.) mg/ml and the electrophoretic method 1.69 ± 0.4 mg/ml. The difference was not significant.

Determinations of the total protein content of amniotic fluid were performed by a biuret method (5 15). Bile pigments in amniotic fluid (ΔE_{450}) were analysed according to the method described by Liley (19).

All patients were blood grouped and screened for antibodies by the routine methods of the hospital blood centre. Patients found to be isoimmunized were tested for maternal antibody titres at regular intervals (papain method, indirect Coombs test) and were also followed with routine liver function tests (serum bilirubin, alkaline phosphatases, thymol turbidity transaminases). Serum creatinine was also determined regularly in all isoimmunized patients. Infants of isoimmunized mothers had haemoglobin and bilirubin determined in their cord blood by the routine methods of the hospital central laboratory.

RESULTS

Mean values for the parameters studied were calculated for 2 week periods. The 9 patients sampled during labour were found to have values within the same range as the other normal patients so these groups were combined.

The following expressions were used to denote statistical significance: probably significant ($0.05 > p > 0.01$) significant ($0.01 > p > 0.001$) and highly significant ($0.001 > p$).

β_2-microglobulin in amniotic fluid

The mean values showed a rapid increase during the second trimester. The increase from the 19th–20th week to the 23rd–24th week was probably significant (Table I Fig. 1). During the later phase of normal pregnancy a gradual decline of

Table I. *Content of β microglobulin in amniotic fluid and maternal plasma during the second trimester of normal pregnancy*

Albumin content of amniotic fluid. Ratio of β_2-microglobulin to albumin in amniotic fluid. Means ± S.E.M. number of samples

Week of pregnancy	β_2 micro in amniotic fluid		β_2 micro in maternal plasma		Albumin in amniotic fluid		$\frac{\beta_2 \text{ micro amn. fl.}}{\text{Albumin amn. fl.}} 10^n$	
		μg/ml		μg/ml		mg/ml		
13–14	4	4.53 ±0.95	3	1.53 ±0.23	3	1.50 ±0.45	3	4.16 ±1.14
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19–20	9	9.33 ±0.65	11	1.24 ±0.09	9	3.52 ±0.39	9	2.88 ±0.32
21–22	2	12.90 —	2	1.45 —	2	3.65 —	2	3.41 —
23–24	5	12.88 ±1.16	5	1.56 ±0.19	5	3.78 ±0.50	5	3.61 ±0.90

the mean values was observed (Fig. 2). The decrease from the weeks 23–24 to the weeks 35–36 was highly significant. The further decrease to the end of pregnancy was not significant. No significant correlation existed between the amniotic fluid levels of β_2-microglobulin and the corresponding levels in maternal plasma during the second ($n = 30$) or the third trimester ($n = 35$). No significant correlation existed between the amniotic fluid levels and the levels in cord serum of infants delivered within 7 days of amniocentesis ($n = 14$).

Rh-isoimmunized patients with affected fetuses (group A + B) showed probably significantly elevated mean values in the 35th–36th and the 37th–38th weeks compared with the normal patients (Fig. 2). The differences between groups A and B were probably significant only in the 33rd–34th week. Individual values for patients with infants who did not survive fell within the same range as

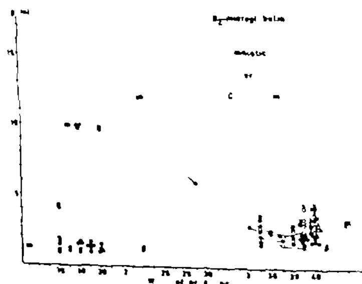


Fig. 1. Individual levels of β_2-microglobulin in normal subjects. Lines connect consecutive values from the same patient.

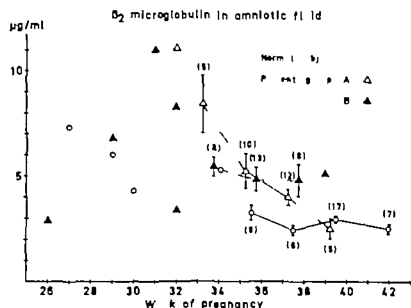


Fig Mean values ± S.E.M. for -week periods with at least 5 samples. Individual levels for other periods. Figures within brackets indicate number of samples.

for patients with surviving infants except for a patient with an hydropic infant who had a value of 8 3 μg/ml as late as in the 37th week. Groups A and B taken together exhibited a significant negative correlation between the β_2-microglobulin levels in amniotic fluid and the cord blood haemoglobin levels for infants delivered within 7 days of amniocentesis ($r = -0\ 59$ $n = 24$) No significant correlation existed with the cord serum bilirubin values for the same groups.

Of the patients with other complications of pregnancy three patients with hepatosis exhibited slightly elevated values compared with the range of normal patients. The patient with polyhydramnios had a value of only 3 8 μg/ml in the 29th week. Otherwise the values fell within the range of normal patients.

In normal patients the amniotic fluid levels did not correlate significantly with infant birth weight or placentar weight for infants delivered within 7 days of amniocentesis ($n = 26$) Taking all categories together 60 patients were delivered within 7 days after amniocentesis Thirty-eight of these patients had values not exceeding 3 50 μg/ml and were all delivered of babies with a birth weight in excess of 2 500 g, 6 of the babies weighed 2 500 to 3 000 g. Of the 22 patients with values above 3 50 μg/ml 4 had babies with a birth weight lower than 2 500 g 13 weighed 2 500–3 000 g.

Table II *Maternal plasma content of β_2 microglobulin*

Means ± S E.M *n* denotes number of samples

Week of pregnancy	Normal patients		Iso-immunized patients group A		Iso-immunized patients group B	
		μg/ml		μg/ml		μg/ml
27 28	1	1 6 —	0	—	0	—
29–30	2	1 75 —	0	—	0	—
31 32	0	—	1	1 9 —	1	1 4 —
33–34	0	—	3	1 90 ±0.21	3	1.53 ±0.15
35–36	6	2.10 ±0.20	6	1 68 ±0.15	7	1 97 ±0.15
37 38	6	2.09 ±0.15	6	1.95 ±0.20	7	1.90 ±0.19
39–40	19	.49 ±0.15	5	1.64 ±0.09	1	1.8 —
41–43	5	2.28 ±0.55	0	—		—

β_2-microglobulin in maternal plasma

In normal patients the mean values were rather constant during the second trimester but showed a slow increase during the third trimester The difference in mean values between the weeks 33–34 and 39–40 was significant (Tables I and II Fig. 1) No significant correlation existed with infant birth weight or placental weight for patients sampled within 7 days prior to delivery ($n = 27$).

Rh-isoimmunized patients group A and B exhibited a tendency to low values compared with the normal patients and for group A the difference was probably significant in the 39th–40th week (Table II). Patients with nonsurviving infants had values in the range 1.1–1.9 µg/ml.

Patients with other complications of pregnancy had values within the range of the normal subjects except a patient with severe diabetes who had the comparatively high value of 3.1 µg/ml in the 36th week.

β_2-microglobulin in cord serum and urine from newborns

All samples of cord serum from normal infants were obtained during the 39th–43rd week. The mean level for these infants was 3.55 ± 0.15 (S.E.M.) µg/ml (range 2.5–4.5, $n = 17$). No significant correlation existed with maternal plasma levels and with infant birth weight or placental weight ($n = 17$).

Only 7 samples were obtained from isoimmunized patients group A and B. The values ranged from 6.0 µg/ml in the 35th week to 3.1 µg/ml in the 39th week. An infant who died of haemolytic disease after delivery in the 33rd week had 3.7 µg/ml.

Samples of the first urine were obtained from nine normal male infants with birth weights ranging 3 080–4 050 g born in the 39th–43rd week. The mean value for β_2-microglobulin was 0.51 ± 0.15 (S.E.M.) µg/ml (range 0.081–1.50). The urine levels did not show any apparent dependency of the levels in amniotic fluid, cord serum and maternal plasma in this small series.

Albumin content of amniotic fluid

Normal subjects exhibited a rapid increase of the mean concentration up to the 19th–20th week and then a slight further elevation up to the 23rd–24th week (Table I). The increase from the 15th–16th week to the 19th–20th week was probably significant. During the later part of normal pregnancy the mean values showed a slow decrease. The difference between the 23rd–24th and the 35th–36th week was highly significant. The further decrease to the end of pregnancy was not significant (Fig. 3).

The relative albumin content showed an increase from $50 \pm 3\%$ (S.E.M.) in the 15th–16th week to $58 \pm$ % of the total protein in the 19th–20th week and $59 \pm 1\%$ in the 23rd–24th week. During the later phase of normal pregnancy the mean values were lower and rather constant, $47 \pm 3\%$ in the 35th–36th week, $51 \pm 3\%$ in the 39th–40th week. The decrease from the 23rd–24th week to the 35th–36th week was significant. The correlation between the albumin and the total protein content was highly significant in the second trimester ($r = 0.97$, $n = 33$) and also in the third trimester ($r = 0.86$, $n = 39$).

Rh-isoimmunized patients group A had a tendency to elevated mean values especially before the 37th week but the differences were not significant (Fig. 3). The relative albumin content of the total protein showed no significant differences between the clinical groups. The group A patients showed a highly significant correlation between the albumin content and the total protein content ($r = 0.87$, $n = 34$) and this was also the case with the group B patients ($r = 0.89$, $n = 36$).

Of the patients with other complications of pregnancy the case of polyhydramnios had a low value of 0.8 mg/ml in the 29th week and one case of hepatosis gravidarum a comparatively high value of 2.2 in the 37th week. Otherwise the values were within the range of normal patients for comparable periods of gestation.

Fifty-four patients of all categories were delivered within 7 days of amniocentesis. Eleven of them had values of 0.90 mg/ml or lower and all these were delivered of babies with a birth weight exceeding 3 000 g. Of the 16 patients with values in the range 0.91–1.10 mg/ml, one had a baby with a weight lower than 2 500 g, 8 weighed 2 500–3 000 g. Of the 27 patients with values exceeding 1.10 mg/ml four had babies with a birth weight lower than 2 500 g.

Relationship between β_2-microglobulin and albumin levels in amniotic fluid

During normal pregnancy in the second trimester the mean ratio between β_2-microglobulin and albumin showed no significant trend (Table I). In the later phase of normal pregnancy the ratio was lower and rather constant (Table III). The decrease from the 23rd–24th to the 39th–40th week was probably significant.

The isoimmunized patients in groups A and B usually exhibited higher ratios to albumin than normal patients and for group A the difference was probably significant in the 37th–38th week.

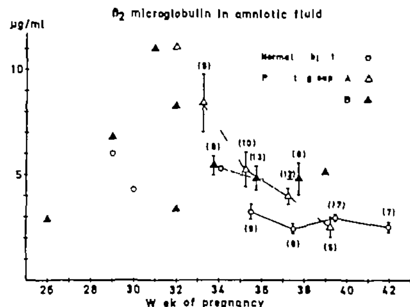


Fig 2 Mean values ± S.E.M. for week periods with at least 5 samples. Individual levels for other periods. Figures within brackets indicate number of samples

for patients with surviving infants except for a patient with an hydropic infant who had a value of 8 3 µg/ml as late as in the 37th week. Groups A and B taken together exhibited a significant negative correlation between the β_2-microglobulin levels in amniotic fluid and the cord blood haemoglobin levels for infants delivered within 7 days of amniocentesis ($r = -0\ 59$ $n = 24$) No significant correlation existed with the cord serum bilirubin values for the same groups.

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Table II *Maternal plasma content of β_2 microglobulin*

Means ± S.E.M denotes number of samples

Week of pregnancy	Normal patients		Iso-immunized patients group A		Iso-immunized patients group B	
	n	µg/ml	*n*	µg/ml	*n*	µg/ml
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29–30	2	1 75 —	0	—	0	—
31 32	0	—	1	1 9 —	1	1 4 —
33–34	0	—	3	1.90 ± 0.21	3	1.53 ± 0.15
35–36	6	2.10 ± 0.20	6	1 68 ± 0.15	7	1.97 ± 0.15
37 38	6	2 09 ± 0.15	6	1.95 ± 0.20	7	1.90 ± 0.19
39–40	19	2.49 ± 0.15	5	1 64 ± 0.09	1	1 8 —
41–43	5	2.28 ± 0.55	0	—		—

β_2-microglobulin in maternal plasma

In normal patients the mean values were rather constant during the second trimester but showed a slow increase during the third trimester The difference in mean values between the weeks 23–24 and 39–40 was significant (Tables I and II Fig. 1) No significant correlation existed with infant birth weight or placental weight for patients sampled within 7 days prior to delivery ($n = 27$).

Rh-Isoimmunized patients group A and B exhibited a tendency to low values compared with the normal patients and for group A the difference was probably significant in the 39th–40th week (Table II). Patients with nonsurviving infants had values in the range 1.1–1.9 µg/ml.

Patients with other complications of pregnancy had values within the range of the normal subjects except a patient with severe diabetes who had the comparatively high value of 3.1 µg/ml in the 36th week.

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Albumin content [illegible] amniotic fluid

Normal subjects exhibited a rapid increase of the mean concentration up to the 19th–20th week and then a slight further [illegible]levation up to the 23rd–24th week (Table I). The increase from the 15th–16th week to the 19th–20th week was probably significant. During the later part of normal pregnancy the mean values showed a slow decrease. The difference between the 23rd–24th and the 35th–36th week was highly significant. The further decrease to the end of pregnancy was not significant (Fig. 3).

The relative [illegible]lbumin content showed an increase from 50 ± 3% (S.E.M.) in the 15th–16th [illegible]eek to 58 ± [illegible] of the total protein in the 19th–20th week and 59 ± 1% in the 23rd–24th week. During the later phase of normal pregnancy the mean values were lower and rather constant, 47 ± 3% in the 35th–36th week, 51 ± 3% in the 39th–40th week. The decrease from the 23rd–24th week to the 35th–36th week was significant. The correlation between the albumin and the total protein content was highly significant in the second trimester (r = 0.97, n = 33) and also in the third trimester (r = 0.86, n = 39).

Rh-isoimmunized patients group A had a tendency to elevated mean values especially before the 37th week but the differences were not significant (Fig. 3). The relative albumin content of the total protein showed no significant differences between the clinical groups. The group A patients showed a highly significant correlation between the albumin content and the total protein content (r = 0.87, n = 34) and this was also the case with the group B patients ([illegible] = 0.89, n = 36).

Of the patients with other complications of pregnancy the case of polyhydramnios had a low value of 0.8 mg/ml in the 29th week and one case of hepatosis gravidarum [illegible] comparatively high value of 2.2 in the 37th week. Otherwise the values were within the range of normal patients for comparable periods of gestation.

Fifty-four patients of all categories were delivered within 7 days of amniocentesis. Eleven of them had values of 0.90 mg/ml or lower and all these were delivered of babies with [illegible] birth weight exceeding 3 000 g. Of the 16 patients with values in the range 0.91–1.10 mg/ml one had a baby with a weight lower than 2 500 g, [illegible] weighed [illegible]500–3 000 g. Of the 27 patients with values exceeding 1.10 mg/ml four had babies with a birth weight lower than 2 500 g.

Relationship between β_2-microglobulin and albumin levels in amniotic fluid

During normal pregnancy in the second trimester the mean ratio between β_2-microglobulin and albumin showed no significant trend (Table I). In the later phase of normal pregnancy the ratio was lower and rather constant (Table III). The decrease from the 23rd–24th to the 39th–40th week was probably significant.

The isoimmunized patients in groups A and B usually exhibited higher ratios to albumin than normal patients and for group A the difference was probably significant in the 37th–38th week.

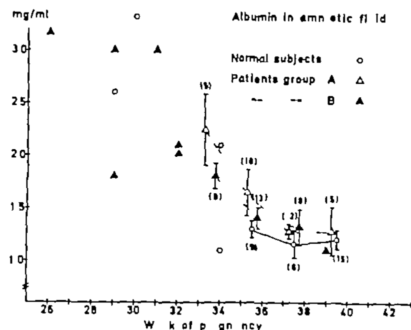


Fig. 3. Mean values ± S.E.M. for -week periods with at least 5 samples. Individual alues for other periods. Figures within brackets indicate number of samples.

For group B the elevation was probably significant both in the 35th–36th and the 37th–38th week. The differences between groups A and B were not significant (Table III).

Table III. *Relations between the content of β_2-microglobulin and albumin in amniotic fluid from the later phase of pregnancy*

Means ± S.E.M. denotes number of samples

Week of pregnancy	Ratio β_2-micro amniotic fl ld	n amniotic fluid/Album in 10³				
	Normal patients		Iso-immunized patients group A		Iso-immunized patients group B	
			n			
27 28	1	1.97 —	0	—	0	—
29–30	1	2.31 —	0	—	1	.27 —
31 32	0	—	1	3 62 —	3	3 10 ±0.71
33–34	2	2.63 —	5	3.87 ±0.24	8	3.23 ±0.31
35–36	9	2.52 ±0.26	10	3.54 ±0.64	13	3 48 ±0.33
37 38	6	2.27 ±0.31	12	3.14 ±0.21	8	3 65 ±0.33
39–40	15	2.63 ±0.21	5	2.14 ±0.41	1	4 64 —
41–43	3	2.23 ±0.18	0	—	0	—

Patients with other complications of pregnancy showed ratios within the range of normal patients except for the patient with polyhydramnios in the 29th week and a patient with hepatosis in the 42nd week who had values above the usual range.

Albumin in amniotic fluid and the ΔE_{450} values

The ratio between the ΔE_{450} values and the corresponding albumin levels was probably significantly elevated for the group A patients compared with the normal subjects in the 33rd–34th

Table IV. *Ratio ΔE_{450}/Albumin mg/ml 100*

Means ± S.E.M. denotes umber of samples

Week of pregnancy	Normal patient		Rh-isoimmunized Group A		Rh isoimmunized Group B	
31 32		—		—	4	8.96 1 82
33–34	4	1 80 ±0 2.	10	3 20 ±0.29	11	5 46 0 61
35–36	11	55 +0 3	14	4 14 0 62	14	7 45 1 73
37 38	9	2 16 ±0 34	14	2 34 ±0 32	9	5 40 1 15
39–40	8	1.26 ±0 28	5	1 74 0 86	1	0 91 —

and 35th–36th week. The group B patients showed a significant elevation compared with the group A patients in the 33rd–34th and 37th–38th weeks (Table IV). For groups A and B taken together the correlation between the ratios and the cord blood haemoglobin values was highly significant for infants delivered within 7 days of amniocentesis ($r = -0.70$, $= 26$). The correlation with the cord serum bilirubin alone was significant ($= 0.56$, $n = 30$). In calculating the ratio the series was expanded with the albumin values from a previous study (17).

Influence of maternal serum creatinine and antibody levels

Taking normal and isoimmunized patients together parallel estimations of β_2-microglobulin in maternal plasma and of maternal serum creatinine were available in 40 cases in the 35th–39th week. No significant correlation between maternal plasma levels of β_2-microglobulin and serum creatinine levels existed for these patients. The mean value of maternal plasma β_2-microglobulin for these patients was 1.99 ± 0.43 (S.D.) µg/ml and of serum creatinine 0.66 ± 0.14 (S.D.) mg/100 ml. The mean values of the laboratories for normal nonpregnant women aged 17–34 was 1.40 ± 0.26 µg/ml and 0.75 ± 0.11 mg/100 ml respectively (11, 33). The difference from these normal values was highly significant for β_2-microglobulin and probably significant for serum creatinine.

Patients belonging to groups A and B showed no significant correlation between the levels of β_2-microglobulin in amniotic fluid and maternal plasma and the corresponding maternal serum titres of specific (anti-D) antibodies ($n = 41$ and 25).

DISCUSSION

Evidence is now accumulating that most of the proteins in amniotic fluid are of maternal origin (1, 6, 14, 28). Immunoglobulin G is found in the urine of the newborn in amounts that make probable higher fetal contribution to the amniotic fluid than the 2 to 5% calculated for other proteins (14). Fetal swallowing is thought to account for 80 to 90% of the protein turnover corresponding to clearance of around 350 ml/day of amniotic fluid and fetal urination at term is calculated to be about 400 ml/day (14). Applying these figures to the present series of patients at term would give a turnover of β_2-microglobulin in amniotic fluid of 800–900 µg/day and an inflow from fetal urine of 200 µg/day. Thus around 25% of the inflow could come from fetal urine. An unknown but perhaps substantial contribution may come from other fetal excretions as β_2-microglobulin is known to occur in saliva in nearly the same concentration as in serum (10). The functional state of the kidney is known to affect the excretion of β_2-microglobulin to a large extent (2, 25, 34). The levels found in the urine of normal babies in the present series are still at least 5 times the normal adult level (11, 25). The high levels observed in amniotic fluid during the second trimester may thus be due to high concentration in the urine from immature fetal kidneys in combination with slow elimination by fetal swallowing. As the fetus becomes more mature the concentration in the urine will decrease and elimination by swallowing will increase. Some direct contribution through the fetal skin is perhaps possible before the 20th week as the skin then has been shown to be more permeable (20, 21).

β_2-microglobulin is rather unique in having a higher concentration in amniotic fluid than in maternal plasma in contrast to most other proteins previously known to occur in amniotic fluid (1, 7, 8, 14, 31). As no active secretory function of the fetal membranes (amnion and chorion) has been shown (1, 20), direct maternal contribution of importance via the membranes against the concentration gradient is not likely. Thus the present series showed no influence from maternal plasma levels. Of interest to note is the poor correlation between cord serum and amniotic fluid levels in the present series which indicates that the process of excretion from the fetus is of much more importance for the amniotic fluid levels than the actual serum concentration.

The distribution of β_2-microglobulin levels in normal amniotic fluid exhibits similar trends as the concentration of albumin and also of total protein (15, 27) with an increase during early pregnancy and a decrease during the last trimester. Because of the gap in the present series the location of the peak period cannot be determined with certainty but the trends indicate that

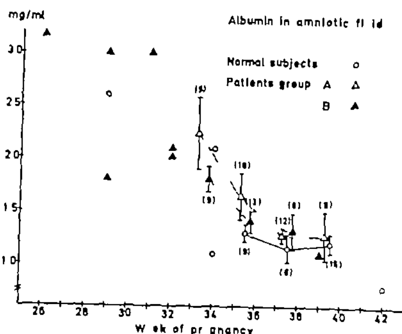


Fig 3 Mean values ± S E.M. for week periods with at least 5 samples. Individual values for other periods. Figures within brackets indicate number of samples.

For group B the elevation was probably significant both in the 35th–36th and the 37th–38th week. The differences between groups A and B were not significant (Table III).

Table III *Relations between the content of β_2-microglobulin and albumin in amniotic fluid from the later phase of pregnancy*

Means ± S.E.M *n* denotes number of samples

Week of pregnancy	Ratio $\beta_2$micr in amniotic fluid/Albumin in amniotic fluid 10^3 Normal patients *n*		Iso-immunized patients group A *n*		Iso-immunized patients group B *n*	
27 28	1	1 97 —	0	—	0	—
29–30	1	2.31 —	0	—	1	2.27 —
31 32	0	—	1	3 62 —	3	3 10 ±0.71
33–34	2	2.63 —	5	3 87 ±0.24	8	3.23 ±0.31
35–36	9	2.52 ±0.26	10	3.54 ±0 64	13	3 48 ±0.33
37 38	6	2.27 ±0.31	12	3 14 ±0.21	8	3 65 ±0.33
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Patients with other complications of pregnancy showed ratios within the range of normal patients except for the patient with polyhydramnios in the 29th week and a patient with hepatosis in the 42nd week who had values above the usual range.

Albumin in amniotic fluid and the ΔE_{450} values

The ratio between the ΔE_{450} values and the corresponding albumin levels was probably significantly elevated for the group A patients compared with the normal subjects in the 33rd 34th

Table IV *Ratio ΔE_{450}/Albumin mg/ml 100*

Means ± S E M *n* denotes number of samples

Week of pregnancy	Normal patients *n*		Rh isoimmunized Group A		Rh-isoimmunized Group B	
31 32		—		—	4	8 96 ±1 02
33–34	4	1 80 +0 22	10	3.20 ±0 29	11	5 46 +0 61
35–36	11	55 +0.21	14	4 14 ±0 6.	14	7 43 ±1 73
37 38	9	16 ±0.34	14	2.34 ±0.32	9	5.40 +1 15
39–40	8	1.6 ±0.28	5	1 74 ±0 86	1	0.91 —

the last weeks of pregnancy correspond very well with those found by other investigators using immunochemical methods (13, 14). The trends observed in the present series were the same as those found previously (17). The results are also in line with previous findings concerning the variations in total protein concentration in normal and Rh-immunized patients (15, 27). The trends observed may have been somewhat influenced by the clinical selection during the last weeks of pregnancy. Even before the 37th week, however, the differences between the groups were not significant. The close correlation with the total protein content also was observed previously (17). Albumin is considered to be the main carrier of bilirubin (23, 32). The ratio between the ΔE_{450} values and the albumin concentrations would thus indicate the degree of saturation with bile pigments. This ratio was clearly elevated in patients with fetal anaemia but seemed to have no better correlation with cord serum bilirubin values than the corresponding ratio calculated from total protein levels (16). Compared with estimations of ΔE_{450} alone the ratio ΔE_{450}/albumin does not seem to give much added information.

As a general conclusion may be said that estimations of β_2-microglobulin in amniotic fluid would be of limited value in the clinical management of iso-immunized pregnant women as it allows no close gradation of fetal involvement. Estimations of β_2-microglobulin and albumin may be of some assistance in evaluating fetal maturity. Further studies of this and perhaps of other low molecular weight proteins may be of value to explain the mechanisms that regulate amniotic fluid protein turnover and the development of fetal renal function during the course of pregnancy.

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REFERENCES

1. Abbas, T. M. & Tovey, J. E. Proteins of the liquor amnii. Brit Med J 1 476, 1960.
2. Berggård, I. & Bearn, A. G. Isolation and properties of a low molecular weight β_2-globulin occurring in human biological fluids. J Biol Chem 243 4095, 1968.
3. Bernier, G. M. & Fanger, M. W. Synthesis of β_2-microglobulin by stimulated lymphocytes. J Immunology 109 407, 1972.
4. Bucht, H. Studies on renal function in man with special reference to glomerular filtration and renal plasma flow in pregnancy. Scand J Clin Lab Invest 3 Suppl. 3, 1951.
5. Bürgi, W., Richterich, R. & Briner, M. UV-photometric determination of total cerebrospinal fluid proteins with a modified biuret reagent. Clin Chim Acta 15 181, 1967.
6. Cederqvist, L. L., Queenan, J. T., Gadow, E. C. & Litwin, S. D. The origin of gamma G globulins in human amniotic fluid. Am J Obstet Gynec 113 838, 1972.
7. Curl, C. W. Immunoglobulin levels in amniotic fluid. Am J Obstet Gynec 109 408, 1971.
8. Derrington, M. M. & Soothill, J. F. An immunochemical study of the proteins of amniotic fluid and maternal and foetal serum. J Obstet Gynaec Brit Comm 68 755, 1961.
9. Doran, F., Gordon, H., Harris, D. A. & Hughes, E. A. Further studies in the assessment of gestational age by amniotic fluid analysis. J Obstet Gynaec Brit Comm 78 603, 1971.
10. Evrin, P.-E., Peterson, P. A., Wide, L. & Berggård, I. Radioimmunoassay of β_2-microglobulin in human biological fluids. Scand J Clin Lab Invest 28 439, 1971.
11. Evrin, P. E. & Wibell, L. The serum levels and urinary excretion of β_2-microglobulin in apparently healthy subjects. Scand J Clin Lab Invest 29 69, 1972.
12. — Serum β_2-microglobulin in various disorders. Clin Chim Acta 1973. In press.
13. Fishbacher, P. H. & Quinlivan, W. L. G. Qualitative and quantitative analyses of the proteins in human amniotic fluid. Am J Obstet Gynec 108 1051, 1970.
14. Gitlin, D., Kumate, J., Morales, C., Noriega, L. & Arevalo, N. The turnover of amniotic fluid protein in the human conceptus. Am J Obstet Gynec 113 632, 1972.
15. Jonasson, L.-E. Total protein content of amniotic fluid from normal pregnancies and from pregnancies complicated by Rh-immunization. Acta Obstet Gynec Scand 51 187, 1972.
16. — The clinical value of amniotic fluid analysis in pregnancies complicated by Rh-immunization or hepatosis. Acta Obstet Gynec Scand 52 113, 1973.
17. Jonasson, L. E. & Hjertén, S. Free zone electrophoresis of amniotic fluid in normal pregnancies and in pregnancies complicated by haemolytic disease. Acta Obstet Gynec Scand 1973. In press.
18. Jonasson, L. E. & Johansson, E. D. B. Progesterone levels in amniotic fluid and plasma from women. II. Levels during pregnancies complicated by Rh-immunization or hepatosis gravidarum. Acta Obstet Gynec Scand 50 345, 1971.
19. Liley, A. W. Liquor amnii analysis in the management of rhesus sensitization. Am J Obstet Gynec 82 1359, 1961.

it cannot be far from the 24th week. The ratios between β_2-microglobulin and albumin are, however, not constant. The reason for this may be that for proteins of higher molecular weights, inflow to the amniotic fluid is mainly through the fetal membranes (1, 14). It is reasonable to assume that this inflow has a better chance to balance elimination from fetal swallowing than the shrinking inflow from the kidneys. Elimination of β_2-microglobulin directly to the mother via the membranes may also play a role in reducing the concentration of β_2-microglobulin more rapidly than for heavier proteins.

The variations in fetal serum concentration of β_2-microglobulin during development is not shown by the present series as it contained only cord sera from term infants. Maternal contribution to the fetal circulation via the placenta seems to be of minor importance at the end of pregnancy as the present study showed no correlation between the maternal and fetal levels. Passage through the placenta would have to work against the concentration gradient. The facts presented so far support the conclusion that β_2-microglobulin in amniotic fluid and fetal serum is largely produced by the fetus itself. The rapid increase in amniotic fluid levels during the second trimester may have some connection with the development of fetal lymphoid tissue which starts around the 8th week (29).

The concentration of β_2-microglobulin in maternal plasma in this series was found to increase during the later part of pregnancy and the levels at term were nearly double the levels previously found in non-pregnant women (11). During the second trimester the concentrations did not differ appreciably from the non-pregnant levels in the same age group (11). The reason for the elevated plasma levels is unclear, but it is not likely that it could be explained by any impairment of maternal renal function as the present series did not contain normal or isoimmunized patients with elevated serum creatinine. The clearance for creatinine and inulin is increased in healthy women during pregnancy (4). In agreement with this, the present study showed that elevated levels of β_2-microglobulin occurred in spite of a low mean creatinine level in maternal serum. The elevated levels during the later phase of pregnancy thus seem to be due to increased maternal production or maybe to a contribution from the fetus.

In isoimmunized patients with affected fetuses (groups A and B) there was a significant correlation with infant cord blood haemoglobin values, but the individual levels of β_2-microglobulin in amniotic fluid showed a considerable overlap between the clinical groups. The trends observed in the present study must be interpreted with some caution as patients with clinical signs of serious fetal disease usually were not allowed to continue their pregnancies as long as patients with indications of slight fetal disease. This selection would tend to minimize the differences between the groups during the last weeks of pregnancy. In the present series maternal influence upon amniotic fluid levels was found to be insignificant. The increased levels of β_2-microglobulin in amniotic fluid do not seem to be due to alterations in the general protein concentration as the ratio to albumin was also increased. A diminution of fetal swallowing would increase the total protein concentration in proportion (14). Disturbance of fetal renal function by the haemolytic process cannot be excluded, but would rather tend to affect the infants with serious disease more than was observed in the present study. Amniotic fluid creatinine levels in patients with fetal haemolytic disease have not been found to differ significantly from the levels in normal patients and this is against any important derangement of renal function as an effect of haemolytic disease (9, 26). Thus it seems most likely that the elevated levels of β_2-microglobulin in amniotic fluid are due to an increase of fetal production. Parallels may perhaps be drawn with intrauterine stimulation of fetal immunoglobulin formation seen in cases of intrauterine infections (29). Further studies of cord sera are required to clarify whether the fetal levels really are elevated in cases of haemolytic disease. The tendency to relatively low levels of β_2-microglobulin in the maternal plasma of isoimmunized patients is hard to explain. If the fetal contribution to the maternal levels is substantial one would rather have expected increased maternal levels in women with fetal haemolytic disease.

Estimation of the amniotic fluid albumin content by radial immunodiffusion resulted in somewhat lower values than those observed in a previous study (17). The difference was not significant and may be due to the immunodiffusion method being more specific. The values for

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THE "PREGNANCY ZONE" PROTEIN AND FETAL WELFARE

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Abstract The "pregnancy zone" protein (PZ) was demonstrated in the sera of 311 out of 350 pregnant women at term (88.9%). The lack of demonstrable PZ was studied in relation to parameters reflecting fetal maturity (birth weight, placental weight and gestational age). Birth weight was significantly lower among infants of women lacking PZ, while for placental weight and gestational age no significant correlation was found with PZ. Furthermore no apparent correlation was found between the lack of PZ in maternal serum and maternal age, parity and previous abortion. The results indicate that lack of PZ in the serum of pregnant women at term is a normal phenomenon compatible with normal pregnancy

The pregnancy zone protein (PZ) has been found in the serum of pregnant women (1, 2, 3, 5, 7, 9, 11, 12, 14), women taking oral contraceptive drugs (4, 8, 9, 12) and men under hormonal treatment for prostatic cancer (10, 11) all results which imply that PZ is a steroid-induced protein

In a previous investigation Beckman et al (7), using starch gel electrophoresis, studied the relation between PZ and a series of genetic and perinatal factors. PZ was found in a significantly higher frequency among mothers carrying a fetus of female sex, but showed no significant correlation with factors such as maternal age, parity, abortion history and maturity criteria.

After purification of the protein (14) an immunological method for detection of PZ was developed (9), which was more sensitive than gel electrophoresis. Using the immunological method PZ seemed to appear in a lower frequency among women aborting spontaneously than among women with apparently normal pregnancies of comparable gestation length (6). These results have raised the question whether the lack of PZ at the term of pregnancy in any way is associated with fetal welfare.

The purpose of this investigation was to determine the frequency of women with demonstrable PZ at term of pregnancy and to investigate whether lack of PZ in maternal serum was in any way related to maternal age, parity, abortion history and some factors reflecting fetal welfare.

MATERIAL AND METHODS

Blood samples from 406 women were collected at the time of delivery (7). From this series 350 cases with complete information on the following factors were included in this study: Birth weight, placental weight, gestational age, maternal age, parity and abortion history. The serum samples were re-examined, using the immunological technique (9). All sera were coded and tested blindly. Storage at −20° for long periods has been found not to have any apparent effect on the detectability of the PZ protein.

RESULTS

At the time of delivery 311 out of 350 women (88.9%) had a demonstrable PZ protein in their sera. Table I presents a comparison between the results obtained with the immunological and electrophoretic methods. The number of serum samples with detectable PZ protein was about doubled using the immunological method. Five samples were classified as "present" by the electrophoretic method, but "absent" using the immunological technique. No statistically significant correlations were found between the absence of demonstrable PZ and placental weight or gestational age, but the birth weight was lower ($P < 0.05$) among infants of women lacking PZ (Table II).

20. Lind, T Kendall, A. & Hytten, F E. The role of the fetus in the formation of amniotic fluid. J Obstet Gynaec Brit Comm *79* 289 1972.
21. Lind, T Parkin, F M & Cheyne G A. Biochemical and cytological changes in liquor amnii with advancing gestation. J Obstet Gynaec Brit Comm *76* 673 1969
22. Mancini, G Carbonara A. O & Heremans, J F Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 235 1965
23. Mandelbaum, B., LaCroix, G C. & Robinson, A. R. Determination of fetal maturity by spectrophotometric analysis of amniotic fluid. Obstet Gynec 29 471 1967
24. Peterson, P A., Cunningham, B. A., Berggård, I & Edelman, G. M. β_2-microglobulin—a free immunoglobulin domain. Proc Nat Acad Sci US *69* 1697 1972.
25. Peterson, P A., Evrin, P E. & Berggård, I Differentiation of glomerular tubular and normal proteinuria Determinations of urinary excretion of β_2-microglobulin, albumin and total protein. J Clin Invest *48* 1189 1969
26. Pitkin, R. M & Zwirek, S. J Amniotic fluid creatinine Am J Obstet Gynec *98* 1135 1967
27. Queenan, J T Gadow E. C., Bachner P & Kubarych, S. F Amniotic fluid proteins in normal and Rh-sensitized pregnancies. Am J Obstet Gynec *108* 406, 1970.
28. Ruoslahti, E., Tallberg, T & Seppälä, M. Origin of proteins in amniotic fluid. Nature *21.* 841, 1966.
29. Smith, T H. Development of fetal and neonatal immunological function. *I* Biology of gestation (ed. N S. Assali) vol. II p. 3 1 Academic Press, New York and London, 1968.
30. Smithies, O. & Poulik, M D Initiation of protein synthesis at an unusual position in an immunoglobulin gene Science *175* 187 1972.
31. Usategui-Gomez M Hopkins, M S. & Fernandez de Castro, A. Serum proteins in amniotic fluids in erythroblastosis fetalis. Obstet Gynec *36* 865 1970.
32. Watson, D Mackay E. V & Trevella, W Amniotic fluid analysis and foetal erythroblastosis. Clin Chim Acta *1* 500, 1965
33. Wibell, L. & Björsell-Östling, E. Endogenous creatinine clearance in apparently healthy individuals determined by 4 hour ambulatory urine collection. Upsala J Med Sci *78* 43 1973
34. Wibell, L., Evrin, P E. & Berggård, I Serum β_2-microglobulin in renal disease Nephron 1973 In press

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REFERENCES

1. Afonso, J. F. & de Alvarez, R. R.: Amer J Obstet Gynec 86, 815, 1963.
2. — Amer J Obstet Gynec 89 204, 1964.
3. Afonso, J. F. & Farnham, N. G.: Amer J Obstet Gynec 84 199, 1962.
4. de Alvarez, R. R. & Afonso, J. F.: Penn Med J 70, 43, 1967.
5. Beaton, G. H., Arroyave, G. & Flores, M.: Am Journ Clin Nutrition 14 269, 1964.
6. Beckman, L., Beckman, G., Magnusson, S. S. & von Schoultz, B.: Acta Obstet Gynec Scand (in press).
7. Beckman, L., Beckman, G. & Stigbrand, T.: Hum Hered 20 530, 1970.
8. Beckman, L., von Schoultz, B. & Stigbrand, T.: Acta Obstet Gynec Scand 50 369, 1971.
9. — Acta Obstet Gynec Scand 52 157, 1973.
10. — Urological Research (in press).
11. Cooper, D. W.: Nature 200, 892, 1963.
12. Margolis, J. & Kenrick, K. G.: Aust J Exp Biol Med Sci 47 637, 1969.
13. Smithies, O.: Advances Protein Chem 14 65, 1959.
14. von Schoultz, B. & Stigbrand, T.: Acta Obstet Gynec Scand 52 51, 1973.

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Table I *Detectability of PZ with the immunological and electrophoretic methods*

(+) = present, (−) = absent

		Immunological method		
		(−)	(+)	*n*
Electrophoretic method	(−)	34	168	202
	(+)	5	143	148
Total		39	311	350

Table II *PZ in relation to birth weight placental weight and gestational length*

	PZ		
	Absent M±S.E.	Present M±S.E.	Significance of difference
Birth weight	3 345±78 g	3 505±28 g	0.05
Placental weight	545±15 g	550± 6 g	0.8 P 0.7
Gestation length	279.4±[illegible]1 days	280.0±0.6 days	0.8 P 0.7

Mothers lacking PZ did not differ with respect to age (Table III) parity (Table IV) and abortion history (Table V)

DISCUSSION

The pregnancy zone protein could be demonstrated in almost 90 of pregnant women at term Development of this α-globulin seems to be a normal phenomenon during pregnancy In this study as in previous reports some individuals were lacking measurable amounts of PZ Women lacking PZ did not differ with respect to age or parity which has been reported in a previous investigation (5).

There was no statistically significant excess of women with previous single or multiple abortions among the mothers lacking PZ. The general impression is therefore that the lack of PZ in the serum of pregnant women at term is a phenomenon compatible with normal pregnancy

It seems that PZ is not produced by some pregnant women Also among women taking oral contraceptive drugs (7 8) and men under hormonal treatment for prostatic cancer a certain fraction of individuals do not react The present information on the lack of PZ during pregnancy can be interpreted as follows. Lack of PZ *at term of pregnancy* may be due to an inability of the mother to produce PZ, while the lack of PZ *in early pregnancy* say the 9–12 weeks of gestation, may be due to one of two different mechanisms (*a*) inability to produce PZ or (*b*) the provocative effect of a normal pregnancy is lacking (6) If this interpretation is correct the lack of PZ in early pregnancy is more informative than at term.

The 39 infants of women lacking PZ were reported healthy at birth without visible malformations and all but two had normal Apgar scores (between 8 and 10 measured 1 and 10 minutes after deliv ery) They had however a somewhat lower birth weight Thus the results of this investigation indicate that absence or low levels of PZ in the maternal circulation at term is a phenomenon compatible with normal pregnancy although some slight effect on fetal development cannot be excluded.

Table III *PZ protein and maternal age*

	PZ present M±S.E.	PZ absent M±S.E.
Maternal age	[illegible]5.95±0.73	[illegible]6.14±0.29
Number examined	311	39

Table IV *PZ protein in relation to parity*

	PZ present	PZ absent	Total
Parity	*n* %	*n*	
0	146 (46.9)	[illegible] (56.4)	168 (48.0)
1	103 (33.1)	10 (25.6)	113 ([illegible])
2	46 (14.7)	6 (15.4)	5 (14.9)
3 and more	16 (5.1)	1 (2.6)	17 (4.9)
No. examined	311	39	350

Table V *PZ and previous abortion*

No. of earlier abortion	PZ present	PZ absent	Total
	n %		*n*
0	274 (88.1)	33 (84.6)	307 (87.7)
1	29 (9.3)	3 (7.7)	32 (9.1)
2 and more	8 (2.6)	3 (7.7)	11 (3.1)
No examined	311	39	350

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RETURN OF OVULATION DURING THE POSTPARTUM PERIOD

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Abstract. The occurrence of ovulation was determined in 21 healthy women after full term delivery by quantitation of the serum progesterone, basal body temperature and cervical mucous ferning tests. The basal body temperature was recorded daily starting during the first week postpartum and serum progesterone estimations and cervical mucus ferning tests were performed at weekly intervals starting after the first month postpartum. All patients were followed until the first postpartum menstruation, and thereafter 18 women continued the study till the second menstruation. The women investigated breast-fed their infants for periods ranging between one and 14 weeks. Ovulation during lactation occurred in none of the cases. After weaning, when menstruation was resumed, ovulation occurred less frequently in patients who lactated for a short period as compared to those who lactated for relatively longer. When the duration of postpartum amenorrhoea was short ovulation preceded the first menstruation less frequently than as occurs with relatively longer postpartum amenorrhoea. The incidence of ovulation before either the first or the second menstruation was lower in primiparae than in multiparae. None of 12 women aged 25 years or less ovulated before the first menstruation while 7 ovulated before the second menstruation. In women above 25 years of age ovulation occurred in 6 out of 9 before the first menstruation and in 5 out of 6 before the second menstruation. The basal body temperature and the cervical mucous ferning tests were equally adequate to detect ovulation as the serum progesterone concentration.

One of the essential functions of the ovaries is to produce a healthy ovum capable of fertilization. Ovulation during the fertile period of the female occurs every month. After full term delivery or abortion, however, a period of anovulation occurs. The length of this period, resumption of ovulation and its relation to the first menstruation have been the subject of many investigations.

Ovulation during lactation amenorrhoea was detected as early as the sixth week postpartum (5, 12). Udesky (16) reported an incidence of 1.5% for ovulation during lactation amenorrhoea and 14% in women who had their first menstruation while still nursing regularly. According to David (2), Grunberger (6) and Parvati (11) the first postpartum menstruation was frequently anovulatory, the second was usually ovulatory and the third was nearly always ovulatory. The three authors reported different incidences of ovulation in relation to menstruation following parturition. Sharman (13) concluded from an extensive study of ovulation after delivery that no ovulation occurred before the first six weeks postpartum, from 7–12 weeks 56% of women ovulated and from 13–24 weeks 86% ovulated. Recently an extensive review of the subject was reported by Sharman (14).

The methods used to detect ovulation in all the above mentioned investigations were either the histologic appearance of the endometrium or the basal body temperature changes. The presence of either a secretory endometrium or a biphasic temperature was considered as evidence of ovulation. These two methods, as are most of the other parameters utilized to detect ovulation, are indirect. Estimation of blood progesterone would be a more accurate method to diagnose ovulation and a functional corpus luteum.

The aim of the present study was twofold: first to detect ovulation during the postpartum period, as judged by the serum progesterone concentration and to find out the effect of lactation, duration of the postpartum amenorrhoea, parity and age on resumption of ovulation, secondly to evaluate the accuracy of the basal body temperature and the cervical mucous ferning as indicators of ovulation as compared to the serum progesterone levels.

the second menstruation ovulation was detected in 12 out of 18 cases. In primiparae, ovulation occurred in 2 out of 10 before the first menstruation and in 5 out of 9 before the second menstruation. In multiparae, on the other hand, ovulation occurred in 4 out of 11 before the first menstruation and in 7 out of 9 before the second menstruation (Table III).

Effect of age

The women studied were divided into two groups according to age: one group of 12 women aged 25 years or less and another group of 9 women aged more than 25 years. In none of the first group did ovulation precede the first menstruation, whereas the second menstruation was preceded by ovulation in 7 out of 12 cases. In the second group ovulation preceded the first and the second menstruations in 6 out of 9 and in 5 out of 6 women respectively (Table IV).

Accuracy of the basal body temperature and the cervical mucous ferning

During 39 cycles ovulation occurred in 18 cycles, according to the serum progesterone concentration. The serum levels of progesterone ranged between 10.5 and 19 ng/ml in 12 estimations and between 7 and 10 ng/ml in 6 estimations.

Table II *Effect of the duration of the postpartum amenorrhoea upon ovulation*

Figures in parentheses indicate percentages

Duration of post-partum amenorrhoea (weeks)	No of cases	Ovulation before first menstruation	No. of cases	Ovulation before second menstruation
12 or less	8	2 (25)	7	4 (57.1)
More than 12	13	4 (30.8)	11	8 (72.7)

Table III *Effect of parity upon ovulation*

Figures in parentheses indicate percentages

Parity	No of cases	Ovulation before first menstruation	No. of cases	Ovulation before second menstruation
Primiparae	10	2 (20)	9	5 (55.5)
Multiparae	11	4 (36.3)	9	7 (77.7)
Total	21	6 (28.1)	18	12 (66.6)

Table IV *Effect of age upon ovulation*

Figures in parentheses indicate percentages

Age (years)	No. of cases	Ovulation before first menstruation	No. of cases	Ovulation before second menstruation
Women aged 25 or less	12	Nil	12	7 (58.3)
Women aged more than 25	9	6 (66.6)	6	5 (83.3)

Table V *No. of ovulatory cycles detected by progesterone, basal body temperature (B.B.T.) and cervical mucus ferning*

Figures in parentheses indicate percentages

Method of detection	No. of cycles	Ovulatory cycles
Progesterone	39	18
B.B.T	39	16 (88.8)
Cervical mucous ferning	39	16 (88.8)

The diagnosis of ovulation by the basal body temperature and the cervical mucous ferning was comparable to progesterone in 16 cycles (Table V). Cervical mucous ferning was atypical in two cycles and the biphasic pattern of the BBT was difficult to determine in another two cycles due to an attack of coryza.

DISCUSSION

The period immediately following delivery is traditionally utilized as a safe period. Pregnancy cannot occur until ovulation is resumed. Lactation is considered to postpone the resumption of ovulation and hence to prolong the infertile period.

Lyon & Stamm (10) according to the daily body temperature found that the initial ovulation following parturition in non-lactating mothers occurred at an average of 10.2 weeks, increased slightly to 10.6 weeks in those who lactated 4 weeks and appeared at an average of 17.0 weeks after 3 months of lactation. Griffith & McBride studied 1 women during lactation amenorrhoea by endometrial biopsies and detected ovulation in one case as early as the sixth week postpartum. This was in accord with the results of Rutherford & Mater who reported that it would be impossible for a female to conceive again before

Table I *Effect of the duration of lactation upon ovulation*

Figures in parentheses indicate percentages

Duration of lactation (weeks)	No. of cases	Ovulation before first menstruation	No of cases	Ovulation before second menstruation
4 or less	12	2 (16.6)	10	6 (60)
More than 4	9	4 (44 4)	8	6 (77 7)

MATERIAL AND METHODS

Twenty-one healthy recently delivered females were selected for the study All the women were collected from the postnatal wards of the University Hospital of Uppsala, Sweden. Ten females were primiparae and 11 multiparae. The average age of the group was 26.5 years, with a range of 19 to 36 years. The course of pregnancy was uneventful, terminating in delivery of full term normal infants. No clinical evidence of abnormal endocrine function was present in any of the cases. The menstrual history was normal in all the cases.

Progesterone assay

The first blood samples were obtained at the end of the first month postpartum and thereafter at weekly intervals until the second menstruation. At each visit about 2 ml antecubital venous blood were collected. Blood was left to clot at room temperature. Serum was then separated by centrifugation and kept at −20°C until assayed. The amount f progesterone in the serum samples was quantitated by a rapid competitive protein binding technique previously described by Johansson (8) A concentration of progesterone in the range of the luteal phase of the ovulatory normal menstrual cycle was considered as evidence of ovulation.

Basal body temperature

Every woman studied, before being discharged from the hospital, received a specially designed temperature chart, and was advised to record the following

1 The morning daily temperature at the same time and before rising.

2. Th date of using a new thermometer if this event occurred.

3 The date and the duration of a y abnormal physiological condition, e.g. headache insomnia etc. or a mild intercurrent disease e.g. common cold etc.

4 The date and th duration of bleeding episodes.

Temperature recording began during the first week postpartum and continued till the second menstruation was resumed. The charts were then collected and the presence of a biphasic shift was recorded.

Cervical mucous ferning

Cervical mucous samples were obtained starting at the end of the first month postpartum. Thereafter each time blood samples were collected for progesterone determination also a cervical mucus specimen was obtained. The cervix was exposed with a speculum and the ectocervix was gently cleaned to carry away vaginal debris and old mucus. A suction syringe with a sterile glass nozzle as utilized to obtain a clean mucus sample from the endocervix. The mucus was then spread on a dry clean glass slide and left to dry at room temperature. The smears were examined under the low power of the light microscope and graded as positive negative or atypical. The changes from positive to negative ferning were recorded.

RESULTS

The duration of the postpartum period, defined as the time between delivery and resumption of menstruation ranged between 6 to 24 weeks. All the patients studied lactated for a period ranging between 1 and 14 weeks. All the women were followed till resumption of menstruation. Thereafter two females could not continue the study and one was excluded as she used hormonal contraceptives after the first menstruation. Accordingly 18 women were followed till the second menstruation was resumed.

Effect of lactation

None of the women studied ovulated during lactation. After weaning when the menstruation was resumed, only 2 out of 12 women who lactated for 4 weeks or less ovulated while 4 out of 9 who lactated more than 4 weeks ovulated. Before the second menstruation, ovulation occurred in 6 out of 10 who lactated 4 weeks or less while it occurred in 6 out of 8 who lactated more than 4 weeks (Table I)

Effect of the duration of the postpartum amenorrhoea

The women were divided into two groups, according to the duration of postpartum amenorrhoea one group with a duration of 12 weeks or less and another group with a duration of more than 12 weeks. In the first group ovulation occurred in 2 out of 8 before the first menstruation and in 4 out of 7 before the second menstruation. In the second group 4 out of 13 women ovulated before the first and 8 out of 11 ovulated before the second menstruation (Table II).

Effect of parity

In 21 women investigated ovulation preceded the first menstruation only in 6 cases while before

cillary use. According to Sharman on the other hand, the reliability and intelligence of the patients in reading and recording the temperature on waking involves a large personal factor which may lead to mistakes. Moreover Diddle (3) pointed out that under certain abnormal physiological conditions the cyclic fluctuations may fail to appear or cannot be determined. Johansson et al. (9) could demonstrate monophasic basal body temperature in women with a normal pattern of progesterone during the luteal phase.

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REFERENCES

1. Cronin, T. J. Influence of lactation upon ovulation. Lancet, London 2 422, 1968.
2. Davis, M. E. The clinical use of oral basal temperatures. J Amer Med Ass *130* 929 1946.
3. Diddle, A. W. Relation of intermenstrual symptoms and signs to ovulation as determined by basal body temperature. Amer J Obst Gyn *56* 537 1948.
4. Elsner P. Erste Menstruation nach Partus und Abortus. Wien Klin Wschr *60* 433 1948.

   Griffith, L. S. & McBride, W. B. L. C. Clinical uses of endometrial biopsy. J Michigan Med Soc *38* 1064, 1939.

6. Gronberger V. Die erste Blutung nach Geburt und Fehlgeburt. Wien Klin Wschr *60*, 498, 1948.
7. Hartman, C. G. C. Science and the Safe Period. Williams & Wilkins, Baltimore 1962.
8. Johansson, E. D. B. Progesterone levels in peripheral plasma during the luteal phase of the normal human menstrual cycle measured by a rapid competitive protein binding technique. Acta Endocr (Kbh) *61* 592, 1969.
9. Johansson, E. D. B., Larsson-Cohn, U. & Gemzell, C. Monophasic basal body temperature in ovulatory menstrual cycles. Amer J Obst Gyn *113* 933, 1972.
10. Lyon, R. A. & Stamm, M. J. The onset of ovulation during the puerperium. J Calif Med *65* 99 1946.
11. Parvati, K. M. Ovulation during lactation as determined by endometrial biopsy. J Obst Gyn India *11* 113 1960.
12. Rutherford, R. N. & Mezer J. Regeneration of the uterine mucosa after spontaneous abortion and normal delivery. J Amer Med Ass *119* 124 1942.
13. Sharman, A. Ovulation after pregnancy. Fertil Steril *2* 371 1951.
14. Sharman, A. The Reproductive Physiology of the Postpartum Period. 1st ed. Livingstone, London, 1966.
15. Topkins, P. C. The histologic appearance of the endometrium during lactation amenorrhea and its relationship to ovarian function. Amer J Obst Gyn *45* 48, 1943.
16. Udesky, I. C. Ovulation in lactating women. Amer J Obst Gyn *59* 843, 1950.

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the sixth week postpartum. Sharman on the other hand observed the first ovulation in a lactating woman at the 13th week postpartum

It appears that ovulation is suppressed during lactation and that the shorter the duration of lactation the less will be the number of ovulatory cycles after weaning. In the present study neither ovulation nor menstruation was resumed in any of the women during lactation After weaning however the number of ovulatory cycles, when the menstruation was resumed, was greater for women who lactated more than 4 weeks than for those who nursed 4 weeks or less. These results confirm earlier studies done by Topkins (15) who found that in the amenorrhoeic period of lactation ovulation did not occur According to Lyon & Stamm the mothers who menstruated during lactation frequently also ovulated and non-menstruating lactating women very rarely ovulated.

The number of ovulatory cycles seems to be related to the duration of the postpartum amenorrhea We found that when the duration of the postpartum amenorrhoea was 12 weeks or less menstrual bleeding was less frequently associated with ovulation than when the duration of the postpartum amenorrhoea was more than 12 weeks. This might point to the fact that the earlier menstruation was resumed the less likely it was to be preceded by ovulation This finding is in accordance with the results of Lyon & Stamm who found that a menstruation of an ovulatory type regularly precedes ovulation in the early puerperium and less frequently in the late puerperium. Elsner (4) investigated the endometrial biopsies obtained from 60 postpartum patients and reported that the sooner the first menstruation reappeared the less frequently was it preceded by ovulation

The first menstruation was less frequently associated with ovulation than the second menstruation. The incidence of ovulation was 28 1% and 66 6% before the first and the second menstruations respectively Moreover women who ovulated before the first menstruation always ovulated also before the second menstruation. This implies that the first cycle was uncommonly preceded by ovulation but when ovulation occurred it became established and recurred regularly in the following cycles. The relation between ovulation and menstruation following parturition was previously studied by Davis, Grunberger and by Parvati Davis according to the daily body temperature found that the first bleeding was rarely ovulatory but the second period was associated with ovulation in approximately half the cases. Grunberger examined the endometrium in 34 postpartum patients and found that one third of patients ovulated in the first cycle while the second cycle was ovulatory as a rule and the third cycle was always ovulatory Parvati found that in lactating mothers the incidence of ovulation before the 1st 2nd and the 3rd menstruations was 16% 50% and 83% respectively

The pattern of ovulation in relation to menstruation after parturition in primiparae was similar to that of multiparae. In both groups ovulation less frequently preceded the first than the second menstruation. However the incidence of ovulation in primiparae before the first and second menstruations was 20% and 55.5% whilst in multiparae it was 36.3% and 77 7% The number involved in this study is too small to make the difference statistically significant. It is possible that the inhibiting effect of pregnancy on the hypothalamus disappears earlier in multiparae than in primiparae As regards the relation between age and ovulation during the postpartum period, we found that the first menstruation was preceded by ovulation in none of the women aged 25 years or less while in women aged more than 25 years the first menstruation was associated with ovulation in two thirds. It may be suggested that the effect of the factors that suppress ovulation persists longer in the younger age group than in the older age group.

Basal body temperature and cervical mucous ferning are among the simple clinical tests that have been used for a long time in the field of obstetrics and gynaecology In the present study ovulation was detected by basal body temperature and cervical mucous ferning and compared to those detected by quantitation of serum progesterone The accuracy of each of the two tests was 88 8% as compared to progesterone This was in agreement with Hartman (7) who found that the basal body temperature correlates well with other indices of ovulation and since it can recorded daily the time of ovulation can be recognized with considerable accuracy Cronin (1) ported that the basal body temperature contr to other methods, is eminently suitable for do

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COMPARISON BETWEEN QUINESTROL AND DIETHYLSTILBESTROL FOR THE INHIBITION OF LACTATION

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Abstract. The effects on the inhibition of lactation of quinestrol (one single tablet of 4 mg) and diethylstilbestrol (5 mg 3 times daily for 5 days) were compared in a controlled clinical trial. During the hospital stay of 5 days breast tenderness was more frequent among the patients who took quinestrol. During the first month at home quinestrol treatment gave less milk secretion and considerably less breast tenderness than diethylstilbestrol. A series of antithrombin III determinations in plasma was too small for definite conclusions to be drawn.

Estrogens are generally considered effective for the inhibition of lactation following late abortion or childbirth. Commonly used drugs, such as diethylstilbestrol or ethinyl estradiol, are short acting and must be taken two or more times daily for about a week. Quinestrol, the 3-cyclopentyl ether of ethinyl estradiol, is a compound which is stored by the body fat and released slowly to give a sustained effect. A single dose of 4 mg by mouth has been found to inhibit lactation completely in about 90% patients (1, 9, 10). A double-blind trial comparing a single dose of quinestrol with the pro-estrogen chlorotrianisene, 12 mg daily for 5 days, showed that discomfort was much more common among the patients given chlorotrianisene, on the 4th day post partum (7).

Inhibition of lactation is associated with an increased incidence of thrombosis (3). Although a causal relationship to estrogen medication is hard to establish because of the many clinical variables involved (4, 9, 12), an increased level of coagulation factor IX has been demonstrated in women using diethylstilbestrol post partum (2). Antithrombin III is a coagulation factor known to fluctuate with the use of estrogen-containing oral contraceptives (6), and it is also influenced by diethylstilbestrol treatment post partum (7).

In our department diethylstilbestrol has been used to inhibit lactation. A drug which could replace it by being taken as one single tablet would represent an obvious advantage. Therefore we have compared the clinical effects of quinestrol and diethylstilbestrol in a controlled trial. Serial determinations of antithrombin III were also performed.

MATERIAL AND METHODS

The patients were women in whom inhibition of lactation was planned. The indications are given in Table I. By random allocation, the patients were divided into two groups, the quinestrol (Q) group comprising 23 women, and the diethylstilbestrol (S) group comprising 18 women. Some clinical characteristics are presented in Table I, which shows homogeneous distribution between the groups.

Quinestrol was given as one tablet of 4 mg initially (day 0), and, if necessary, one additional tablet on day 4. A second tablet was given to 6 of 23 patients.

Diethylstilbestrol was given as one tablet (5 mg) 3 times daily for 5 days.

The medication was to start as soon as possible after delivery. Practical difficulties often entailed a time lag, the median number of hours from delivery to the first tablet being 13.5 in the Q group and 10.0 in the S group. Normally the patients stayed in hospital for 5 days post partum. If additional estrogen medication was required when they returned home, diethylstilbestrol was given, regardless of the initial medication.

In hospital the clinical condition was assessed daily by the same observer. An important criterion of treatment success is the absence of breast tenderness, but this can hardly be graded objectively. Upon leaving the hospital each patient received a questionnaire with a few specific questions about milk secretion, breast pain, additional estrogen medication, and whether symptoms suggestive of mastitis and thrombosis were experienced. All the patients

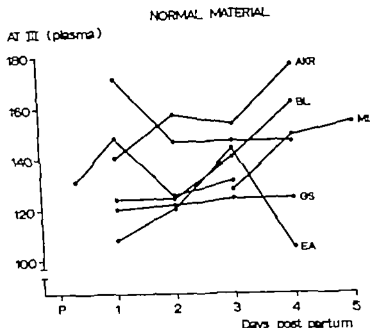


Fig. 1 Antithrombin III concentrations in plasma in group of lactating omen following normal deliveries.

patients lie in the same range a those of the control patients There is a wide scatter in all the groups and no obvious trend with time post partum The only tendency is that the values seem to be lower in the S group compared to the Q group (Fig. 2). It should be recalled that it is the ery low alues of antithrombin III which carry increased risk of thrombosis.

DISCUSSION

The results indicat that diethylstilbestrol inhibits lactation more rapidly than quinestrol whereas quinestrol is better after the first few days At home the patients ho took quinestrol had less milk secretion and considerably less breast tenderness, while those taking diethylstilbestrol often experienced rebound effect This is compatible with the fact that quinestrol has a depot effect, hich is said to be maintained for t least six week after single dose (10)

The advantage of one single tablet of quinestrol over five-day course of diethylstilbestrol is obvious Quinestrol-treated patients experience more breast tenderness during the first few days, but in hospital this can be relieved by supportive measures and analgesics. Brown & Snell (1) stated that mild discomfort on the fifth or sixth day after 4 mg quinestrol generally subsided without further treatment. We ga e an additional tablet in such cases, but this could possibly have been omitted. In our trial the time from delivery to start of treatment was often too long. The sooner after delivery estrogen is given the more likely is it to inhibit breast activity (4), and six hours may be a practical time limit (10). Simple instructions may prevent unnecessary delays.

In Norway all mothers are encouraged to breast feed their babies. Immediate post partum inhibition of lactation is therefore seldom indicated. The number of candidates for such treatment will necessarily be small, even in a large obstetrical department. The indications for lactation inhibition in the present material were mainly late abortion and fetal death. The results may not be directly transferable to communities where lacta tion inhibition in mothers who ha e live babies is more common. The comparison of results between the two treatment groups is not thought to be influenced by the high number of pathological pregnancies, however

I this small trial the incidence of thrombosis and the analyses of antithrombin III are inconclusive. It should be pointed out, however that the only pparent difference was that antithrombin III levels in the S group were lower

Table I *Clinical characteristics in the quinestrol (Q) and diethylstilbestrol (S) groups*

Treatment group	Q	S
Late abortion or fetal death	20	15
Missed abortion	0	1
Previous mastitis	1	0
Adoption	2	2
Total indications	23	18
Para I	12	8
Para II	7	3
Para III+	4	7

but two (one in the S and one in the Q groups) returned the forms after one month at home

Antithrombin III was determined in plasma samples from 4 patients in the Q group, 5 patients in the S group, and from 7 control patients wh were lactating post partum. The plasma samples were stored at −20°C and later sent collectively by plane to Oslo in the frozen state. They were examined by Dr Magne K. Fagerhol, Ullevål Hospital, Oslo, by the single radial immunodiffusion method (5). Antithrombin III concentrations are xpressed as the percentage f normal serum concentrations. As *about 30% of the antithrombin III is consumed during* coagulation, normal values in plasma from non-pregna t women re about 150%.

RESULTS

Evaluation of treatment

Table II shows that there was an even distribution of milk secretion between the two treatment groups in hospital. The general impression was that secretion was heavier among the patients treated with quinestrol. At home the incidence rose in the S group and remained stationary in the Q group. The resulting difference borders on significance if one uses a one-sided test ($P=0.05$).

In hospital, breast tenderness (Table III) was

Table II *Comparison of effects. Milk secretion*

In hospital the distributio was homogeneous. At home m lk secretion was more frequent n the S group $P=0.05$ in a one-sided fourfold table test

	In hospital				At home			
	Q		S		Q		S	
	No.	%	No.	%	No.	%	No.	%
Present	13	56.6	11	61.1	13	59.1	15	88.2
Absent	10	43.4	7	38.9	9	40.9		11.8
Total	23	100.0	18	100.0	22	100.0	17	100.0

Table III *Comparison of effects. Breast tenderness*

In hospital breast tenderness was more frequent among Q treated patients, $P=0.05$ in a one-sided fourfold table test. At home it was significantly more frequent in the S group, $P=0.01$ in a two-sided test

	In hospital				At home			
	Q		S		Q		S	
	No.	%	No.	%	No.	%	No.	%
Present	9	39.1	2	11.1	6	27.3	13	76.5
Absent	14	60.9	16	88.9	16	72.7	4	23.5
Total	23	100.0	18	100.0	22	100.0	17	100.0

prevalent among the quinestrol-treated patients, the trend being completely reversed at home, where pain was experienced by 76.5% of those given diethylstilbestrol against only 23.5% of those who had quinestrol. This difference is highly significant, $P=0.01$ in a two-sided test. As judged from these results, diethylstilbestrol seems to have a better immediate symptomatic effect than quinestrol but this lasts only as long as the tablets are taken while the action of quinestrol seems to increase with time. This impression was substantiated by the need for additional estrogen medication at home. Four of 23 patients in the Q group and 10 of 18 in the S group took tablets at home

The evaluation of the consistency and color of the breasts while the patients were in hospital revealed a slight tendency towards harder consistency among those given quinestrol (not tabulated)

Complications

One patient (Q) developed thrombophlebitis in hospital 3 days post partum (2 days after the first quinestrol tablet). She was treated with anticoagulants, and later given another quinestrol tablet without recurrence of the symptoms.

Another patient (S) reported bilateral leg pain the legs feeling warm and swollen after she had left the hospital

A third patient (Q) reported bilateral leg pain at home but there was no swelling.

There were no cases of mastitis in either of the groups.

Antithrombin III investigations

Comparing Figs. 1 and 2, we see that the antithrombin III-concentrations of the estrogen treated

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VALUE OF DETERMINATION OF F D P DURING PREGNANCY BY IMMUNOCHEMICAL AND LATEX AGGLUTINATION INHIBITION METHODS

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Abstract In toxaemia, hepatosis, placental insufficiency and other complications of pregnancy fibrin deposits occur in the kidney liver and placenta. When broken down these products appear in the blood as fibrin degradation products (F D P) The value of repeated immunochemical determinations of F D P was investigated in the serum of 705 pregnant women F.D.P in concentrations of 5 to 40 µg ml were found in 78% patients who later developed clinical complications, particularly toxaemia and hepatosis Further a rapid latex-agglutination-inhibition test was evaluated At concentrations of F.D.P above 15 µg ml complete agreement was found between the results of this test and those of the immunochemical method

On proteolytic digestion of fibrinogen/fibrin by the enzyme plasmin degradation products (F D P) appear in the blood These products, which are of different molecular weights, can be determined with immunochemical methods F.D P occur in diseases with associated systemic fibrinolysis intravascular coagulation with secondary fibrinolysis, as well as on local deposition of fibrin with secondary fibrinolysis.

In healthy non-pregnant and pregnant women the blood contains only small amounts of F D P (< 5 µg ml) In complicated pregnancy however the concentration of F D P is increased (1 10) Of 1 001 women examined on one occasion during the latter part of pregnancy Hedner & Astedt (5) found that the F D P in the serum was increased (10–30 µg/ml) in 99 and that 90 of these had some complication, usually toxaemia, hepatosis or urinary tract infection.

This paper concerns the value of repeated immunochemical determinations (8) for the F D P during pregnancy and the evaluation of a new quick latex-agglutination-inhibition test.

MATERIAL AND METHODS

The hospital records of 705 pregnant women in whom the F.D P had been routinely determined, usually 6, 4 and 2 weeks before parturition were analysed. The average number of F.D.P determinations per patient was 2 6.

Notes were made of any increase in F.D P during pregnancy as well as any subsequent complications in the mothers or in the infants.

Toxaemia was said to be present in pregnant women who had at least 2 of the following 3 symptoms: oedema requiring treatment, proteinuria, and hypertension (> 140/90 mmHg). The diagnosis of hepatosis required itching and increased serum transaminase (SGOT and SGPT, > 100 units) urinary tract infection, positive culture of urine and clinical symptoms

Determinations of F.D P in 1 051 samples by an immunochemical method (8) and a new quick latex-agglutination-inhibition test were compared.

Collection of blood. Blood was collected in tubes containing an inhibitor of fibrinolysis, E aminocaproic acid (EACA) as well as thrombin to prevent in vitro fibrinolysis and incomplete coagulation with residual fibrinogen (30 NIH units thrombin and 25 mg EACA to 3 ml blood). Serum from these samples was prepared in the way described by Paraskevas et al. (9).

Determination of F.D.P 1) Immunochemical method (8). In this method an antiserum against the D-fraction of the F.D P is applied to agarose gel. With high voltage electrophoresis serum migrates into the gel. If F.D P are present, they will produce precipitation peaks. The height of such peak is measured and related to a standard of high molecular weight degradation products. The method measures F.D P down to a concentration of 5 µg ml. In the presence of epsilon aminocaproic acid and thrombin this method will not show any F D P in the serum of healthy women.

2) *Latex-agglutination-inhibition test for determination of F.D P* (Fig. 1) Equal volumes of antifibrinogen (prepared in rabbit and purified by AB Kabi, Stockholm, Sweden) and serum were mixed in a test tube and left at room temperature for at least 3 min. 0.1 ml of this mixture was then placed on a black glass plate together with 0.1 ml of a latex suspension (latex particles coated

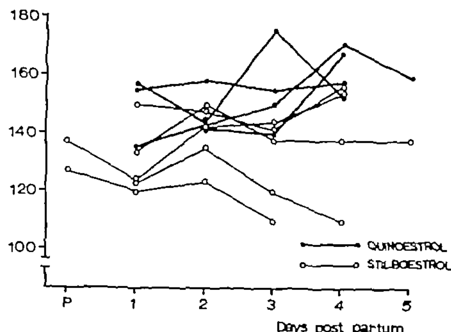


Fig Antithrombin III concentrations in plasma in unselected groups of women who received quinestrol or diethylstilbestrol post partum.

than those in the Q group. Gjønnæss & Fagerhol (7) found that in diethylstilbestrol-treated women antithrombin III concentration did not return to normal non pregnant levels but remained low on the fifth day post partum which suggests a strong estrogenic action. These observations are compatible with our clinical results, which showed that the initial response to diethylstilbestrol was stronger than to quinestrol To establish a definite relationship between antithrombin III concentra tions and estrogen medication would require a larger series, and observations extending into the second week when possible differences due to the depot effect might be revealed.

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REFERENCES

1 Brown, D & Snell, M Inhibition of lactation with quinestrol. Brit Med J 3 326, 1968
2 Daniel, D G Bloom, A. L., Giddings, J C Campbell, H. & Turnbull, A. C. Increased factor IX levels in puerperium during administration f diethylstilboestrol. Brit Med J 1 801 1968
3 Daniel, D G Campbell, H & Turnbull, A. C. Puerperal thromboembolism and suppression of lactation. Lancet 287 1967
4 Jeffcoate T N A., Miller J Ross, R. F & Tindall, V R. Puerperal thromboembolism in relation to the inhibition f lactation by oestrogen therapy Brit Med J 4 19 1968.
5. Fagerh l, M K. & Abildgaard, U Immunological studies on antithrombin III. Scand J Haemat 7 10, 1970
6. Fagerhol, M K., Abildgaard, U Bergsjø, P & Jacobsen, J H Oral contraceptives and low antithrombin III concentration. Lancet 1175, 1970
7 Gjønnæss, H. & Fagerhol, M. Plasma concentrations of antithrombin III in untreated and estrogen-treated puerperal women Abstract Volume II Congre s, The International Society on Thrombosis and Haem stasis, Oslo, Norway July 14th–16th, 1971 p. 20.
8. King, M K. A comparative study of two fat-stored oestrogens in inhibiting lactation. Brit J Clin Pract 26 .67 1972.
9 Millar D G & Littlepage B N C Puerperal thromboembolism Lancet 1 887 1970
10. Roberts, S. Inh bition of lactation Estrovis (Quinestrol 4 mg) copious fluids for 48 hours. Mid-Chron Nurs Notes 83 72, 1970.
11 Scholz, H. R. Therapeutische Inhibition und Suppression der Laktation mit Quinestrol. Med Welt 72 19, 1971
12. Stewart, K. S Kerridge D F & Dennis, K. J Suppression of lactation. Brit Med J 49 1969

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Table II. *Comparison of results of examination of 1051 samples for F.D.P. by immunochemical and agglutination-inhibition methods*

ml	Immunochemical method (No. of samples)	Latex method, No. of samples: Without F.D.P.	With F.D.P.
1–5	945	936	9
6–10	80	63	17
13	16	8	8
16	10	0	10
Total	1 051	1 007	44

approaches that found by Hedner & Astedt (5) in women examined once during the last two months of pregnancy. This does not, however, mean that it is unnecessary to determine the F.D.P. repeatedly since F.D.P. were found also in 11% apparently normal pregnancies. Moreover, in most of these cases F.D.P. were demonstrable in only single test. In those cases where F.D.P. were demonstrable on only one occasion they probably reflected a transient complication, e.g. minor fibrin deposits or microthrombosis in the placenta (2, 3), which could be coped with by the fibrinolytic system. No clinical complications had occurred and delivery was normal. Repeated determinations of the F.D.P. are therefore useful for estimating the course of an incipient complication of pregnancy.

The comparison between the immunochemical method for determination of F.D.P. (8) and the latex-agglutination inhibition test used by us showed good agreement. In the present investigation the test failed to detect the lowest concentration of F.D.P. (15 µg/ml), but it showed complete agreement in the patients with F.D.P. concentration of more than 15 µg/ml. This corroborates the findings of Mefliger (7), that the lowest concentration of F.D.P. that can be determined by his direct latex agglutination test is 1 µg/ml. The method described in this paper thus seems to be at least as sensitive as Mefliger's (7). The quick and simple latex-agglutination test for demonstrating F.D.P. thus proved to be a valuable method for routine screening in antenatal care.

The investigation thus demonstrated the value of repeated F.D.P. determinations during pregnancy to detect and estimate the course of complications.

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REFERENCES

1. Bonnar, J., Davidson, J. F., Pidgeon, C. F., McNicol, G. P. & Douglas, A. S.: Fibrin degradation products in normal and abnormal pregnancy and parturition. Brit Med J 3: 137, 1969.
2. Davis, B., Jeremson, R. F. & Langley, F. A.: Significance of placental pathology in transplacental haemorrhage. J Clin Path 21: 322, 1968.
3. Fox, H.: White infarcts of the placenta. J Obstet Gynaec Brit Comm 70: 980, 1963.
4. Hedner, U.: Urinary fibrin(ogen) degradation products (FDP) in uraemia and after renal transplantation. Scand J Clin Lab Invest 1973.
5. Hedner, U. & Astedt, B.: Fibrinolytic split products in serum and urine in pregnancy. Acta Obstet Gynec Scand 49: 363, 1970.
6. Marder, V. J. & Shulman, N. R.: High molecular weight derivates of human fibrinogen produced by plasmin. I. Physicochemical and immunological characterization. J Biol Chem 244: 2111, 1969.
7. Mefliger, E. J.: Detection of fibrinogen degradation products by use of antibody coated latex particles. The possibilities and limits of the method. Thrombos Diathes Haemorrh 23: 211, 1970.
8. Nilsson, I. E.: Separation and estimation of split products of fibrinogen and fibrin in human serum. Thrombos Diathes Haemorrh 18: 487, 1967.
9. Paraskevas, M., Nilsson, I. M. & Martinsson, G.: A method for determining serum inhibitors of plasminogen activation. Scand J Clin Lab Invest 14: 138, 1962.
10. Wardle, E. N. & Menon, I. S.: Fibrinolysis in pre-eclamptic toxemia of pregnancy. Brit Med J 2: 625, 1969.

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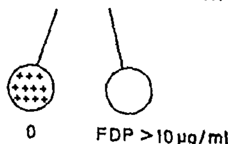


Fig. 1. Agglutination-inhibition test for FDP

with fibrinogen by AB Kabi, Stockholm, Sweden). The plate was gently rocked for min and the agglutination was estimated by visual inspection. F.D.P. if any in the serum sample tested will be neutralised by the antifibrinogen during the incubation time and no antiserum will be left to agglutinate the latex particles. On the other hand, if the serum contains no F.D.P. the antifibrinogen will not be neutralised during the incubation and will then induce agglutination of the fibrinogen coated latex particles. The minimum amount of F.D.P. demonstrable by the test can be varied by adjustment of the amount of antifibrinogen incubated with the serum sample. In the present investigation the test was adjusted to inhibit agglutination when the sample contained F.D.P. in concentration above 10 µg/ml. Reliable results require adjustment of the antiserum concentration to each latex suspension. The reproducibility of the assay was found to be good both when high molecular weight fibrinogen degradation products [purified according to Marder & Shulman (6)] and D-products [purified according to Nilehn (8)] were used in a concentration of 5 µg/ml. But the method could not detect E-products [purified according to Hedner (4)] in concentrations up to 20 µg/ml.

In an additional preliminary investigation neither serum nor urine from 45 apparently healthy persons inhibited agglutination. 1 062 samples (840 serum samples and 222 urine samples) from patients with various diseases were tested simultaneously with the latex agglutination inhibition test and the immunochemical method of Nilehn. The frequency of discordance between the results obtained with the two methods was 6.4%.

Table I. Complications with or without F.D.P. in 705 pregnant women

Figures within parentheses denote percentages

Diagnosis	No. of cases With F.D.P.	Without F.D.P.
Hepatosis	21 (72)	8
Toxaemia	37 (75.5)	12
Urinary tract symptoms	4 (100)	0
Diabetes mellitus glycosuria	6 (86)	1
Abruptio placentae (acute F.D.P.)	3 (75)	1
Miscellaneous	9 (100)	0
Normal pregnancy	70 (11)	533
Total	150	555 705

RESULTS

Clinical complications had been noted in the records of 102 of the 705 patients. In 80 (78%) of these F.D.P. had been found in serum in a concentration of 5 to 40 µg/ml.

The clinical complications and the frequency of F.D.P. are given in Table I. The miscellaneous group includes 3 with infection (pneumonia in 2 and rubella in 1), 3 with obscure abdominal pain, one with Rh immunisation requiring exchange transfusion of the infant, one with ulcerative colitis and one with maternal bleeding cerebral aneurysms, which required neurosurgical intervention after delivery by caesarean section.

F.D.P. were demonstrated in 11% patients in whom pregnancy had been uncomplicated.

The results of the 1 051 comparative determinations of F.D.P. in the serum by the immunochemical method and the latex agglutination inhibition test are summarised in Table II.

DISCUSSION

It is well documented that an increased amount of F.D.P. in the serum during pregnancy is indicative of a complication of pregnancy especially toxaemia (1, 5, 10). It is due to the breakdown of fibrin deposits in the kidney, liver and placenta.

Repeated examinations revealed F.D.P. in 78 patients with clinical disorders. This figure ap-

ble II. Comparison of results of examination of 51 samples for F.D.P. by immunochemical and agglutination-inhibition methods

/ml	Immunochemical method (No of samples)	Latex method. No of samples	
		Without F.D.P.	With F.D.P.
5	945	936	9
10	80	63	17
15	16	8	8
16	10	0	10
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responds to that found by Hedner & Astedt (5) in women examined once during the last two months of pregnancy. This does not, however, mean that it is unnecessary to determine the F.D.P. repeatedly since F.D.P. were found also in 11 % apparently normal pregnancies. Moreover in most of these cases F.D.P. were demonstrable in only a single test. In those cases where F.D.P. were demonstrable on only one occasion they probably reflected a transient complication, e.g. minor fibrin deposits or microthrombosis in the placenta (2, 3), which could be coped with by the fibrinolytic system. No clinical complications had occurred and delivery was normal. Repeated determinations of the F.D.P. are therefore useful for estimating the course of an incipient complication of pregnancy.

The comparison between the immunochemical method for determination of F.D.P. (8) and the latex-agglutination inhibition test used by us showed good agreement. In the present investigation the test failed to detect the lowest concentration of F.D.P. (< 15 μg/ml), but it showed complete agreement in the patients with F.D.P. concentration of more than 15 μg/ml. This corroborates the findings of Melliger (7), that the lowest concentration of F.D.P. that can be determined by his direct latex agglutination test is 17 μg/ml. The method described in this paper thus seems to be at least as sensitive as Melliger's (7). The quick and simple latex-agglutination test for demonstrating F.D.P. thus proved to be a valuable method for routine screening in antenatal care.

The investigation thus demonstrated the value of repeated F.D.P. determinations during pregnancy to detect and estimate the course of complications.

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REFERENCES

1. Bonnar, J., Davidson, J. F., Pidgeon, C. F., McNicol, G. P. & Douglas, A. S.: Fibrin degradation products in normal and abnormal pregnancy and parturition. Brit Med J 3: 137, 1969.
2. Dem, B., Jeanson, R. F. & Langley, F. A.: Significance of placental pathology in transplacental haemorrhage. J Clin Path 21: 322, 1968.
3. Fox, H.: White infarcts of the placenta. J Obstet Gynaec Brit Comm 70: 980, 1963.
4. Hedner, U.: Urinary fibrin(ogen) degradation products (FDP) in uraemia and after renal transplantation. Scand J Clin Lab Invest 1973.
5. Hedner, U. & Astedt, B.: Fibrinolytic split products in serum and urine in pregnancy. Acta Obstet Gynec Scand 49: 363, 1970.
6. Marder, V. J. & Shulman, N. R.: High molecular weight derivatives of human fibrinogen produced by plasmin. I. Physicochemical and immunological characterization. J Biol Chem 244: 2111, 1969.
7. Melliger, E. J.: Detection of fibrinogen degradation products by use of antibody coated latex particles. The possibilities and limits of the method. Thrombos Diathes Haemorrh 23: 211, 1970.
8. Nilehn, J. E.: Separation and estimation of split products of fibrinogen and fibrin in human serum. Thrombos Diathes Haemorrh 18: 487, 1967.
9. Parmikevas, M., Nilsson, I. M. & Martinsson, G.: A method for determining serum inhibitors of plasminogen activation. Scand J Clin Lab Invest 14: 138, 1962.
10. Wardle, E. N. & Menon, I. S.: Fibrinolysis in pre-eclamptic toxemia of pregnancy. Brit Med J 2: 625, 1969.

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ISOLATION AND IDENTIFICATION OF CORYNEBACTERIUM VAGINALE (HAEMOPHILUS VAGINALIS) IN WOMEN WITH INFECTIONS OF THE LOWER GENITAL TRACT

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Abstract. The occurrence of Corynebacterium vaginale (Haemophilus vaginalis) and other micro-organisms in the cervix of healthy women and women with lower genital tract infections (LGTI) was studied. C. vaginale was isolated from 31.4% of 70 patients with LGTI but not from any of 28 healthy controls. In 5.7% of the patients with LGTI, C. vaginale was the only organism recovered from the cervix. In the patients with LGTI the symptoms and signs did not vary with the presence or absence of C. vaginale. LGTI with C. vaginale could not be diagnosed with certainty from findings in wet mounts, Gram or Papanicolaou stained smears. C. vaginale was not isolated from any of the women in whom the vaginal smears revealed flora with predominance of bacilli morphologically of Doderlein's type. The study also included evaluation of methods for isolating and identifying C. vaginale. In vitro C. vaginale was sensitive to all antibiotics tested with the exception of sulphonamides.

Corynebacterium vaginale previously known as Haemophilus vaginalis, has been widely discussed as a common cause of infections of the lower genital tract (LGTI) in females (3, 4, 7, 9, 13, 14, 16, 20). Only a few Scandinavian investigations of C. vaginale have been published (2, 21). Despite numerous studies, the role played by C. vaginale in LGTI is still uncertain. In most studies the criteria used for identifying C. vaginale has been rather vague. However recently new methods for isolating and more strict criteria for identifying C. vaginale have been described by Dunkelberg et al. (5, 6).

This study concerns the occurrence of C. vaginale in cervical specimens of healthy women and of women with LGTI. Signs and symptoms in patients harbouring C. vaginale are described. Culture techniques and methods for identifying C. vaginale including the use of gas chromatography are discussed. The diagnostic value of wet mounts, Gram and Papanicolaou stained smears in the evaluation of LGTI with C. vaginale is appraised. The in vitro susceptibility of C. vaginale to antibiotics is considered.

MATERIAL AND METHODS

Clinical material

The series consisted of 98 non-pregnant, non-puerperal women in the child-bearing age. Most of them were in the age range 17 to 30. Seventy of the women had symptoms and signs of infection of the lower genital tract, while the remaining 28 women had no such symptoms or signs. All the women were sexually active and none had received antibiotic treatment before the examination.

Women with infection of the lower genital tract (LGTI). The 70 women in this group all complained of increased vaginal discharge and most of them had additional symptoms such as foul-smelling discharge and itching or a sensation of burning in the vulva. Examination revealed pathological vaginal secretion, reddening of the vaginal mucosa and purulent secretion from the cervical os.

Non-infected controls. None of the 28 women in this group had any symptoms referable to infection of the genital tract. Examination revealed normal whitish, creamy vaginal content and normal mucus from the cervical os. Most of these patients had sought advice on contraception or a varied health check-up. The age distribution and socio-economic status of the infected and non-infected women are similar.

Collection and transport of samples

Specimens for isolation of C. vaginale, Neisseria gonorrhoeae, other bacteria and mycoplasmas were collected

from the cervix. For the isolation of N gonorrhoeae samples were also collected from the urethra and the rectum The cervical os and the urethral orifice were exposed by the insertion of a sterile speculum into the vagina before the performance of any other diagnostic procedure The specimens were collected with sterile cotton tipped swabs treated with charcoal, which were rotated in the cervical canal. A modified Stuart s me dium (SBL-medium) was used as transport medium (11). In addition to this medium, Proteose Peptone No. 3 (Difco) (5) and haematin agar (18) slopes were used in some instances. Cultures of the samples were started within 4 hours of collection.

W t mounts Gram and P panicolaou tained smears

Discharge from the posterior vaginal fornix was mixed with a drop of saline and immediately examined as a wet mount under a light microscope. Material collected from the cervical canal the portio and the posterior vaginal fornix was fixed in ethanol and stained according to Papanicolaou. Cervical secretion was Gram stained. The wet mounts and the smears were examined par ticularly for clue cells i.e. epithelial cells covered with numerous coccoid bacteria (3 9 15). Gram and Papa nicolaou stained smears were also studied for the occur rence of bacilli of Doderlein s type. Infestation with Trichomonas vaginalis was diagnosed if the wet films contained mobile trichomonades. Clinical infection with Candida albicans was diagnosed from findings of reddened vaginal mucosa with whitish patches and the occurrence of "pseudohyphae in wet films.

Isolation procedures

Isolation of C vaginal The cervical specimens were in oculated onto pepton-starch-de trose (PSD) gar (Difco) prepared according to Dunkelberg et al (5) nd onto solid Casman medium containing 5% sheep blood. The cultures wer incubated for 48 hours at 37°C in boxes insufflated with a gas mixture of 90% N and 10% CO The PSD agar plates were ead nder a stereo-microscope Bacteria suspected to be C aginale were Gram stained After being passaged once on f esh PSD agar suspected colonies of C vaginale were inoculated into PSD broth containing glucose maltose and starch. Tests were made for catalase acti ity and growth-inhibition by H_2O (5).

In order to establish the presence f the cervical specimens of other bacteria as w ll cultures were also made on blood agar plates (18), which ere incubated in 10% CO

Isol tion of Mycoplasma hominis and N gonorrhoeae The medium used for isolation of M hominis consisted of Heart-infusion aga (Difco), horse serum, yeast, penicillin nd thallium acetate (18). N gonorrhoea cul-tured on a medium described by Re (22), containing 25 IU/ml of polymyxin and 3 µg/ml of trimetoprim.

Test f the growth f C vaginal on som add tional media

In addition to th media mentioned bove isolated strains of C. ginale were inoculated onto horse blood agar (18) plates containing 5% sheep blood and into tryptose and thioglycollate broth. Growth was also tested on the mycoplasma medium described above, but omitting penicillin and thallium acetate as well as on the gonococcal medium.

Gas chrom tography of vol til fatty acids produced by C. vaginale grown in PSD medium

Gas-liquid analysis was performed with a Hewlett Packard gas chromatograph model 5750, equipped with a hydrogen flame ionization detector (FID) and fitted with a Perkin Elmer pen-recorder model 165 A glass column, 3 ft long and with an outer diameter of 1/8th inch, packed with Carbowax 20M and terminated with Chromosorb W (Hewlett Packard) was used. The column was operated isothermally at 110°C. The flow rate of the carrier gas, N was 60 ml/min. The flow rate of H through the FID was 35 ml/min and that of air 900 ml/min. A range of 10² and an attenuation of for the FID were used

Colonies of C. vaginale were scraped off the surface of PSD agar plates after incubation at 37°C for 48 hours. Extractions of volatile fatty acids were made according to the methods described in Anaerobe Laboratory Manual (12). PSD broth as well as scraps of uninoculated but incubated PSD agar plates ere used as standards" A volume of 0.5 µl of the sample to be examined was injected into the instrument.

The volatile fatty acid peaks were identified by comparing the retention times with those of highly purified standards (British Drug Houses). The composition of the standard solution used was the same as that described in the Anaerobe Laboratory Manual" (12).

Antibioti susceptibility of C vaginale

Ten of the isolated strains of C. vaginale were tested by the disc method (8) for susceptibility of antibiotics. The tests were made in petri dishes, 9 cm in diameter containing 20 ml of PSD agar The tests wer read after the cultures had been incubated for 48 hours at 37°C The different antibiotic discs (AB Biodisk Stockholm) used are given below

RESULTS

The isolation of C vaginale and other micro-organisms from the cervix of patients with and without LGTI

The number of women with C vaginale M hominis, T vaginalis, N gonorrhoeae and with clinical infection with C. albicans in the 98 patients studied is shown in Table I C. vaginale was isolated from 31 4% of the 70 women with LGTI but not from any of the 28 healthy con trols 5 7 % of the patients with LGTI were found to harbour C. vaginale only and none of the other organisms mentioned above. M hominis was recovered from 44.2% of the women with

Table I. *Incidence of Corynebacterium vaginale, Mycoplasma hominis, Trichomonas vaginalis, Neisseria gonorrhoeae and clinical infection with Candida albicans in 70 women with signs of genital infections (LGTI) and in 28 women with no such signs*

Group	No. of subjects (N)	Organisms isolated: C. vaginale	M. hominis	T. vaginal.	N. gonorrhoeae	C. albicans
LGTI	70	22	31	11	1	5
No clinical signs of genital infection	28	9	2	0	0	0

LGTI and from 7.1% of the controls. From 81.8% of the patients harbouring C. vaginale, M. hominis was also isolated. T. vaginalis was found in the wet mounts from 15.7% of the patients with LGTI and from 4.5% of those with C. vaginale. N. gonorrhoeae was isolated from one of the patients with signs of infection but not from any of the controls. Clinical signs of infection with C. albicans were found in 7.1% of the LGTI patients. In the cervical cultures from the LGTI patients a predominance of Escherichia coli or coliform rods were found in six cases, Proteus mirabilis in one, and enterococci in seven. No such bacteria were found in the specimens from the 28 controls.

The isolation rate of C. vaginale did not vary with the two transport media, i.e. Proteose Peptone No. 3 and Stuart modified medium, when used for cervical samples from the same subjects. Haematin agar slopes were found to be less reliable for the isolation of C. vaginale.

Clinical findings in patients harbouring C. vaginale

The clinical findings in the LGTI patients from whom C. vaginale was and was not isolated are compared in Table II. The patients harbouring T. vaginalis, N. gonorrhoeae and those with clinical signs of infections with C. albicans are excluded.

In four patients, C. vaginale was the only organism recovered from the cervix. In another 12 cases, C. vaginale and M. hominis were the only micro-organisms isolated. There was no difference in duration of symptoms between these two groups of patients. All 16 women had reddening and oedema of the vaginal epithelium and all complained of an increased and foul-smelling vaginal discharge. Itching and sensation of burning in the vulva were reported by about half of the patients in each group. The four patients harbouring only C. vaginale had a thin and white-grey discharge. In the patients from whom both C. vaginalis and M. hominis were recovered, the discharge had either a thin or a thick homogeneous consistency with a white-grey or a slightly yellow colour. In two patients, one in each group, the discharge was slightly frothy.

Findings in wet mounts, Gram and Papanicolaou stained smears

The wet mounts from all cases in which C. vaginale was isolated contained an increased number of leucocytes. There was no significant difference in the arrangement of the leucocytes in the wet mounts of the patients from whom C. vaginale alone was isolated, as compared with those from whom other micro-organisms were recovered, with or without C. vaginale. "Clue cells" were most easily recognized in the Gram stained smears, but were also found in the wet mounts and in the Papanicolaou stained smears. In the groups of patients with LGTI the frequency of smears containing "clue cells" did not vary with the presence or absence of C. vaginale in the cultures. "Clue cells" were not found in the smears of any of the healthy controls. The Gram stained smears obtained from the patients from whom C. vaginale had been isolated contained either none or at most a few bacilli of Döderlein type.

Characteristics of C. vaginale

The bacteria isolated during the present study and regarded as C. vaginale were gram-negative rods, which when cultured on PSD agar produced white, convex colonies. The colonies had an entire border when inspected under the stereo-micro-

Table II *Symptoms and signs in patients with LGTI harbouring and not harbouring Corynebacterium vaginale*

		C. vaginale	
		Isolated (N = 16)	*Not isolated* (N = 36)
Symptoms			
Duration	<1 month	4	15
	>1 month	12	21
Discharge	Foul-smelling	16	11
Itching	Of the vulva	6	5
Burning	Of the vulva	6	4
Signs			
Discharge			
Colour	White–yellow	6	22
	White–grey	11	14
Consistency	Thin	11	21
	Thick homogeneous	5	15
Frothiness		2	2

Cases with N gonorrhoeae, T vaginalis and with clinical signs of infection with C. albicans were excluded.

Neither did C. vaginale grow on the gonococci medium used in the present study Solid mycoplasma medium supported growth of C. vaginale, but no growth was obtained on this medium when it contained penicillin and thallium acetate.

Gas chromatography analysis of C vaginale

Material from uninoculated but incubated PSD broth and PSD agar plates produced peaks corresponding to acetic propionic, butyric and valeric acid Material from colonies of C. vaginale added mainly acetic acid to the mentioned fatty acids found in pure medium.

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Ten of the isolated strains of C. vaginale which were tested for susceptibility to penicillin G lincomycin, chloramphenicol cephalotin tetracyclines, sulphonamides, ampicillin and streptomycin were all found to be sensitive to these antimicrobials, with the exception of sulphonamides, to which all the strains were resistant

scope They were catalase negative and fermented maltose starch and though weakly glucose. Growth was inhibited by H O on PSD agar They were oxidase negative and did not reduce tellurite Growth was not stimulated by haem or co-enzymes. Staphylococcus aureus did not stimulate growth of C. vaginale when cultured on blood agar plates. The results of the gas chromatography analyses are described below

Growth of C vaginale on various media

The growth of C. vaginale on horse blood agar plates incubated aerobically was very scanty although it was somewhat better when incubation was made anaerobically An abundant growth was found on horse blood agar plates containing 5% sheep blood. The colonies of C vaginale on this medium were grey and not surrounded by any haemolytic zone. C. vaginale also grew fairly well on haematin agar plates incubated in 10% CO_2. The colonies on this medium were grey and uncharacteristic. On Cassman's medium containing 5% sheep blood small "dew-drop" colonies were found which often produced α haemolysis of the medium. The bacteria multiplied in thioglycollate broth, where they formed puff ball colonies" C. vaginale did not grow in tryptone broth.

DISCUSSION

LGTI in women caused by bacteria mycoplasmas, chlamydial agents or viruses, or often mixtures thereof are often diagnosed as non specific vaginitis" But with the improvements in laboratory diagnostic methods during recent years, a more precise diagnosis should often be possible Some authors consider C. vaginale the principle cause of non-specific vaginitis" (2, 9)

In earlier series the isolation rate of C vaginale from the lower genital tract of women has varied from a few to more than 50% In most previous studies of C vaginale either none or only scanty information is given on the clinical findings of genital infection in the women studied In the present material the overall isolation rate of C vaginale was — 4% After classification of the material according to clinical criteria, the recovery rate from the patients with signs of LGTI was 31 4 while C vaginale was not isolated from any of the clinically normal women Apart from differences in the series studied, the variation in the reported isolation rates of C vaginale is certainly also due to differences in the isolation and identification techniques used In many reports the criteria used for the identification of C vaginale have be

Lately Dunkelberg et al. (5) presented more strict criteria for the identification of C. vaginale. These criteria were applied in the present study. The occurrence of C. vaginale in the present series of women with LGTI was about the same as that found by Dunkelberg et al. (7) in a series of female V.D. clinic patients.

Many authors have regarded C. vaginale in the lower genital tract of women as pathogenic (2, 3, 4, 7, 9, 12, 16, 21). Gardner & Dukes (9) inoculated C. vaginale intravaginally in 13 healthy women. Only one of them developed signs of genital infection. In similar experiments Criswell et al. (4) found clinical infections to develop in about one fourth of the women studied. However, the inoculation doses used in these experiments were probably much higher than those which might cause naturally occurring infections.

Of our patients harbouring C. vaginale, this was the only micro-organism found in the cervix in 18.2%. C. vaginale was never isolated from the healthy controls. This might support the concept of a causative role of this organism in LGTI. Furthermore, in the stained vaginal smears of all the women with C. vaginale, either none or at most, a few lactobacilli of Doderlein type were found.

The pathogenicity of C. vaginale, as well as that of many other micro-organisms, *in LGTI* is difficult to assess, because of the simultaneous occurrence of many different organisms in these conditions. For instance, C. vaginale was found together with M. hominis in our material in 37% of the patients with LGTI. Amies & Jones (1) suggested that mycoplasmas found in the genital tract might be L-forms of C. vaginale. But in the light of the present knowledge of these organisms such a relationship can now be refuted.

It has been claimed that C. vaginale is venereally transmitted (7, 9). According to Gardner & Dukes (10) C. vaginale is common in the urethra of men whose wives harbour this organism. In our study the sexual partners were not examined. In a recent study Mårdh & Colleen (19) found a low incidence of C. vaginale in the urethra, in expressate of the prostate and in ejaculate of healthy males as well as of patients with chronic prostatitis.

Gardner & Dukes (9) classified the bacteria discussed in the present study as a member of the genus Haemophilus because it grew on blood agar. Zimmermann & Turner (23) recommended its classification as a Corynebacterium because of its morphology and staining properties. Moss & Dunkelberg (17), on the basis of gas chromatographic analysis, concluded that C. vaginale is not related to Propionibacterium or Butyribacterium. The findings in the present study lend support to this conclusion. In accordance with the latter authors we found acetic acid to be the major volatile fatty acid produced by C. vaginale. Although the taxonomic position and thereby the nomenclature of this bacterium is not definitively established, the name C. vaginale has been accepted in the current presentation.

Dunkelberg et al. (5) recommended a transport medium for C. vaginale consisting of Proteose Peptone No. 3. As the SBL-medium (11) was found to be as useful as the transport medium suggested by Dunkelberg et al., the sampling and transport procedures recommended in Sweden for N. gonorrhoeae may also be used for the isolation of C. vaginale.

The colony morphology was very useful for discerning C. vaginale. Even though haematin agar, Casman's medium and horse blood agar containing 5% sheep blood supported growth of C. vaginale, PSD agar was preferable for the isolation of this bacterium from clinical specimens, since the colony morphology was easily observable on this medium when examined under a stereo-microscope in translucent light.

It has been stated that infection with C. vaginale should be strongly suspected in women with a grey, homogenous and foul-smelling vaginal discharge (3, 9). Our findings did not lend support to such a conclusion. The signs and symptoms in the LGTI patients with and without C. vaginale showed no significant differences (Table II).

The findings in wet films have also been claimed to constitute convincing evidence of the presence of C. vaginale (9). In wet films from patients harbouring C. vaginale a striking feature has been stated to be the paucity of leucocytes (3, 9). However, in the present series there was an increased number of leucocytes in the wet films from all patients harbouring C. vaginale. Gardner & Dukes (9) and Brewer et al. (3) stressed the diagnostic value of the demonstration of "clue cells" in wet films and *Gram* stained

Table II Symptoms and signs in patients with LGTI harbouring and not harbouring Corynebacterium vaginale

		C. vaginale	
		Isolated (N = 16)	Not isolated (N = 36)
Symptoms			
Duration	< 1 month	4	15
	> 1 month	12	21
Discharge	*Foul-smelling*	*16*	*11*
Itching	Of the vulva	6	5
Burning	Of the vulva	8	4
Signs			
Discharge			
Colour	White-yellow	6	22
	White-grey	11	14
Consistency	Thin	11	21
	Thick, homogeneous	5	15
Frothiness		2	2

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scope. They were catalase negative and fermented maltose starch and though weakly glucose. Growth was inhibited by H_2O_2 on PSD agar They were oxidase negative and did not reduce tellurite. Growth was not stimulated by haem or co-enzymes. Staphylococcus aureus did not stimulate growth of C. vaginale when cultured on blood agar plates. The results of the gas chromatography analyses are described below

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LGTI in women caused by bacteria, mycoplasmas, chlamydial agents or viruses, or often mixtures thereof are often diagnosed as non-specific vaginitis But with the improvements in laboratory diagnostic methods during recent years, a more precise diagnosis should often be possible Some authors consider C. vaginale the principle cause of non-specific vaginitis" (.., 9).

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Acta Obstet Gynec Scand 53 91–95 1974

CASE REPORT

UTERINE ARTERIO-VENOUS FISTULA

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Abstract A case of arterio-venous fistula of the uterus is presented The literature is reviewed and the etiology is discussed. Special attention should be paid to the oc currence of previous pregnancy

Arterio-venous (a-v) fistula of the uterus is a very rare condition also known as cirsoid aneurysm, a-v aneurysm and arteriectasia. The etiology of a-v fistula of the uterus and adnexa has never been satisfactorily explained. The cause of a-v fistula of the adnexa seems to be better understood than that of the uterus (1). A study of the literature reveals 11 cases of a-v fistula of the uterus which were treated surgically The etiology of these cases is unknown. A brief presentation is given in Table I.

There is a rather high frequency of previous pregnancies in cases of a-v fistula. Two patients out of 11 were said never to have been pregnant. Two patients had had a hydatidiform mole (14, 16) In all cases except two there was a enlargement of the uterus and generally the uterus was soft and reminiscent of pregnancy The age of the patients varied between 31 and 63 years.

We hav recently seen 19-year-old patient nh a-v fistula of the uterus. The clinical presentation was typical, but the patient was younger than ny case reported earlier

Pre-operativ diagnosis was made by arteriography As these cases are ery rare, we wish to eport it to throw light on the etiology of this condition

CASE REPORT

The patient as 19-year-old primigravida ith history of regular and normal menstruation. In the 15th eek of pregnancy she as admitted to hospital for legal abortion She as given an extraamniotic instillation of 20% sodiumchloride solution She also received L oxytocin and borted. A curettage as then performed. The post-operative course as uneventful and after her first menstrual period the patient was given oral contraceptives. Six months later her menstruation became more profuse and she as given tranexamic acid (Cyclocapron®). A few months later she again had severe bleeding and curettage was performed. Because of the continuous bleeding the patient was given blood transfusions besides tranexamic acid and an estrogen-progesterone preparation (Primosiston®). After this third curettage was performed Microscopic examination of the endometrium did not explain the bleeding and there was no general disturbance of the coagulation mechanism. Prednisolone was added to the treatment but the bleeding continued. During five eeks the patient as given 9 600 ml of plasma and 16.5 litre blood in 33 transfusions. Hysteroscopy showed small bleeding points and few buds of vessels ithin an area of 4 4 mm in the fundus. A pelvic arteriography revealed moderately enlarged uterus with large uterine arteries. Within the myometrium there was rather large arteriovenous shunt The uterine veins ere filled early in the arterial phase suggesting the presence of an a-v fistula. Because of this, hysterectomy as performed. At the operation ide vessels ere seen on the front of the uterus and in the parametrium and the uterus looked pregnant The recovery as uneventful. Immediately after the operation the uterus was opened and barium contrast was injected into the uterine artery and was seen to flow from two vessels hich opened ithin an elevated area in the fundus

PATHOLOGY

Macroscopic finding

A somewhat enlarged regular and smooth uterus. The myometrium was normal in consistency and structure. On the cut surface one could see several dilated vessels. The terine cavity was normal in shape and size but in the fundus there was 4 mm high protrusion cm in diameter This area was bluish because of a great number

smears. Lewis & O Brien (15) found that Papanicolaou stains could be used to identify this organism with an acceptable degree of accuracy The results of the present study did not confirm such a diagnostic value of clue cells in wet films or smears. This discrepancy might be due to the fact that more strict criteria (5 6) were used for the identification of C. vaginale There was no significant difference in the incidence of "clue cells" in the wet films or in the stained smears of the patients with LGTI with or without C. vaginale being isolated. Neither were any clue cells" found in 8 out of 22 patients, from whom C. vaginale had been recovered.

Various forms of local and general antibiotic therapy have been suggested for the treatment of genital infections with C. vaginale. Gardner & Dukes (9) recommended local treatment with triple sulphonamide Lewis et al. (16) suggested ampicillin orally The results of our in vitro antibiotic susceptibility tests of C. vaginale suggest that sulphonamides should not be effective The present study indicates that there is very often a mixed infection in patients harbouring C. vaginale which has to be considered when making a choice of therapeutic agents.

REFERENCES

1 Amies, C. R & Jones, S A A description of Haemophilus vaginalis and its L forms. Can J Microbiol 3 579 1957
2. Bergman, S., Lundgren, K M. & Lundstrom P Haemophilus vaginalis in ginitis. A study of a cocci dominated vaginitis. Acta Obst t Gynecol Scand 44 8 1965
3 Brewer J I Halpern, B & Thomas, G Hemophilus vaginalis vaginitis. Am J Obstet Gynecol 74 834 1957
4 Criswell, B S., Ladwig, C L Gardner H L & Dukes, C. D Haemophilus vaginalis. Vaginitis by inoculation from culture Obst t Gynec 33 195 1969
5 Du kelberg, W E. J Skaggs, R & Kellogg, D S Jr Method for isolation and identification of Coryne bacterium vaginale (Haemophilus aginalis). Appl Microbiol 19 47 1970
6. Dunkelberg, W E J A study and new description of Corynebacterium aginale (Haemophilus vaginalis). Am J Cl Pathol 53 370 1970.
7 Dunkelberg, W E., Jr Skaggs, R., Kellogg, D S Jr & Domescik, G K. Relative incidence of Corynebacterium aginale (Haemophilus aginalis), Neisseria gonorrhoeae and Trichomona spp. among women attending a enereal disease clinic. Brit J Vener Dis 46 187 1970
8. Ericsson, H Tunevall, G & Wickman, K., A paper disc method for determination of bacterial sensitivity for antibiotics. Scand J Clin Lab Invest 12 414 1960
9 Gardner H. L. & Dukes, C. D Haemophilus vaginalis vaginitis. A newly defined specific infection previously classified "non-specific" vaginitis. Am J Obstet Gynec 69 96., 1955
10. — Haemophilus vaginalis vaginitis. Am NY Acad Sci 83 280 1959
11 Gastrin, B & Kallings, L O Improved methods for gonococcal sampling and examination in large scale. Acta Pathol Microbiol Scand 74 36., 1968
12. Holdemann, L. V & Moore W E. C. (Eds.) Chromatographic procedures for analysis of acid and alcohol products. I Anaerobic Laboratory Manual, p. 113 Southern Printing Co., Blacksburg, Virginia, 1972.
13 Kümmel, J Klinische und bakteriologische Untersuchungen uber die unspezifische Scheidenentzündung Arch Gynaekol 199 5 1963
14 Leopold, S. Heretofor undescribed organism isolated from the genito-urinary system. US Armed Forces Med J 4 263 1953
15 Lewis, J F & O'Brien, S. Diagnosis of Haemophilus vaginalis by Papanicolaou smears. Am J Clin Pathol 51 412, 1969
16. Lewis, J F O'Brien, S. M., Ural, U M. & Burke, T Corynebacterium vaginale vaginitis. Review of the literat re and presentation of data based on vaginal cultures from 1 008 patients. Am J Obstet Gynec 112 87 1972.
17 Moss, C W & Dunkelberg, W E., Jr Volatile and cellular fatty acids of Haemophilus vaginalis. J Bacteriol 100 544 1969
18 Mårdh, P A & Westrom, L. Tubal and cervic l cultures in acute salpingitis with special reference t Mycoplasma hominis and T-strain Mycoplasmas Brit J Vener Dis 46 390, 1970.
19 Mårdh, P A & Colleen S (in press).
20 Redmond, D L & Kotcher E Comparison of cultural and immunofluorescent procedures in the identification of Haemophilus vaginalis. J Gen Microbiol 33 89 1963
21 Renkonen, O V Widholm, O & Vartiainen, E., Vaginal infection. I Microbial findings Acta Obstet Gynec Scand 49 Suppl ., 5 1970.
22. Reyn, A Laboratory diagnosis of gonococcal infections. Bull WHO 32 449 1965
23 Zimmermann K & Turner C. G The taxonomic position of Haemophilus aginalis (Corynebacterium vaginale). J Pathol Bacteriol 85 213, 1963

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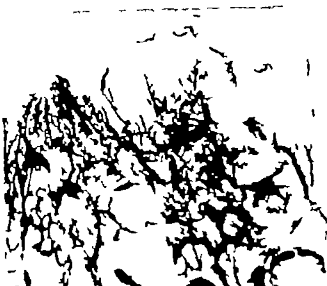


Fig. 1

on can cause a permanent arterio-venous communication in the genital tract (6, 18 21 24).

II *Heredity* Some a-v fistulae are congenital (1 11). This seems however to be more reasonable explanation when the anomaly is located in the adnexa (1 22)

III a *Malignant trophoblastic disease* In cases of malignant trophoblastic disease a fistulae are seen in the uterus and are reported as one of the criteria of the disease (3 5).

III b *Healed malignant trophoblastic disease* Although sub-group of the previous one it is a well defined group with persistent a-v fistulae in the uterus following successful chemotherapy for malignant trophoblastic disease (7 10, 19).

IV *Pregnancy* Necrosis of the chorionic villi may cause an a-v fistula in the uterus (3 4). In an arteriographic study of 112 normal pregnancies one case of a-v fistula was found (3)

Our case of a- fistula in the uterus is unusual as it has been found in rather young woman The pathological change was superficial and in close connection with the uterine cavity where

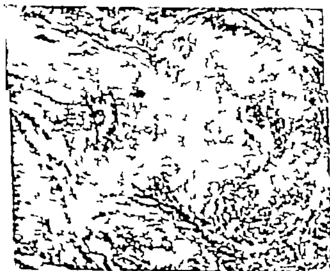


Fig 2

Table I *Published idiopathic cases of a-v fistula of the uterus*

Author	Pub-lished	Age of patient	Preg-nancies	Abortions	Menopause	
Dubreuil et al.	1926	62	4	0	53	Uterus enlarged. Vaginal pulsations. Large vessels seen during operation
Graves et al.	1927	62	4	1	48	Somewhat enlarged uterus. Large tortuous vessels seen during operation
Reynolds et al.	1949	42	1	1	—	Uterus soft and enlarged. Large tortuous vessels seen during operation
Berlin et al.	1952	63	8	2	55	Uterus large as a 6-week pregnancy. No pulsation
Gaines et al.	1955	58	4	3	45	Uterus symmetrically enlarged equal to a 3-month pregnancy. Several large tortuous vessels seen during operation
G rdner et al.	1954	43	0	0	—	Uterus enlarged equal to a 2-month pregnancy. A soft murmur was heard over the uterus prior t operation
Williams	1954	34	0	0	—	Uterus was normal i size but soft and pulsating
Hoge	1955	58	7	3	52	The uterus was somewhat enlarged. Patient operated because of a hea y bleeding following curettage
Liggins	1964	41	4	2	—	Uterus enlarged equal to a 3-month pregnancy. Preoperative angiography revealed a–v changes
Frencken et l.	1965	42	10	2 (one hydatidiform mole)	—	Uterus pulsated and w enlarged equal to a 3-month pregnancy. Preoperative angiography revealed a–v changes
Hibbard et al.	1972	31	0	1 (hydatidiform mole)	—	Uterus normal size. Moderately enlarged uterine essels. A swelling in the uterine wall. Preoperative angiography revealed v changes

of vessels. The appearance was not that of an ordinary polypus. In the centre of this area two vessels opened. The rest of the endometrium was normal. The uterus was cut into 2 mm thick slices and several preparations from various parts of the uterus were made for microscopic study. The protruding area within the fundus was serially cut for microangiographic photos (Fig. 1). This study revealed a great number of irregular vessels varying in size. The vessels showed signs of pathological anastomoses.

Microscopic findings

Most of the myometrium had a normal structure with normal vessels. Within the elevated area the tissue was provided with a large number of vessels most of which were pathologically dilated with irregular lumina. A comparison between the microangiographic pictures and the histological preparations from the same area confirmed the pathological anastomoses between irregularly dilated arteries and thickened arterialized veins (Fi 2). At the histological examination it could b seen that the two vessels opening on the tube surface consisted of ruptured thick and irregula veins with signs of arterialisation. One of the tw vessels opening in the centre of the pathologic area was partly filled with a thrombus (Fig. 3). The conclusion was that there existed a well out lined submucous arterio-venous aneurysm in th uterine fundus.

DISCUSSION

The etiology of a-v fistula of the uterus is no completely established. Certain factors howeve are said to be able to cause this condition.

1 *Trauma*. An injury to adjacent artery and

[illegible] Obstet Gynecol [illegible]

[illegible] fistula. [illegible]

[illegible] S. M. Arteriovenous [illegible] Obstet Gynecol [illegible]

[illegible] & Fineberg, C. [illegible] Am J Obstet [illegible]

[illegible] Arterio- [illegible] JAMA [illegible]

[illegible] Wideman, G. L., Gravlee, L. C. & Jones, W. N. Arteriovenous aneurysm of the uterine artery and vein following total abdominal hysterectomy. Am J Obstet Gynecol 77: 200, 1959.

[illegible] Williams, G. A. Arteriovenous aneurysm of the uterus. Am J Obstet Gynecol 67: 199, 1954.

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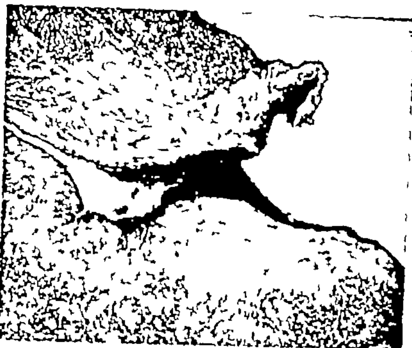


Fig 3

two vessels had ruptured. The significance of the legal abortion is not clear It could have con tributed to the rupture but not to the formation of the a v fistula The time from the abortion to the first symptoms is too short for this. A definite diagnosis of an a v fistula can only be made by arteriography Cases before 1955 are therefore difficult to evaluate as this method was not available.

As the etiology is still uncertain in several cases of a v fistula it is of importance that well docu mented cases are reported so our knowledge of this rare disease can increase Special attention to the occurrence of a previous pregnancy must be made.

REFERENCES

1 Benson, R C Dotter C I Peterson, C G Metcalf J., Bristow J D & K ushaar O F Congenital ar teriovenous fistula nd pregnancy Am J Obstet Gynecol 9 672, 1965

2. Berlin A C & Horwitz, D Arteriectasi of uterine rtery Am J Obst t Gynecol 64 347 1952

3 Borell U & Fernstrom, I Arteriovenous fistulae of the uterus and adnexa Acta Radiol 49 1 1949

4 — Ectopic pregnancy ssociated w th rteriovenous fistula, Acta Obstet Gynecol Scand 38 404 1959

5 Borell, U Fernstrom, I Moberger G & Olsson L The Diagnosis of Hydatidiform Mole Malignant Hydatidiform Mole and Choriocarcinom ith Special Reference to the Diagnostic V lue of Pel ic Arteriography Thomas, Springfield Ill. 1966.

6 Camp, O B Arteriovenous fistula following hysterectomy Am J Surg 86 40 1953

7 Cockshott, W P & de V Hendrickse, J P Persistent arteriovenous fistulae following chemotherapy of malignant trophoblastic disease Radiology 88 329 1967

8 Decker D G Fish, C & Joergens, J Arteriovenous fistulas of th female pelvis. A diagnostic problem Obstet Gynecol 31 799 1968

9 Dubreuil G & Loubat, E. Anévrysme cirsoid de l'utérus Ann Anat P thol 3 697 19 6

10 Fernström, I Personal communication

11 Fontaine R Le Gal, Y Kieny R Bollack, C L & Chaddad, F Cirsoid aneurysm within the framework of arteriovenous fistulae A propos of 25 personal cases. Lyon Chir 63 33, 1967

12 Frencken, V A. M. & Landman G H M Cirsoid aneurysm of the terus: Specific arteriographic diagnosis. Report of a case Am J Roentgenol 95 735 1965

13 Gaines, J & Greenwald, J C. Uterine arteriovenous fistula Am J Obstet G necol 65 997 1953

14 Ga dner H J Cirsoid aneurysm of the uterus Am J Obstet Gynecol 65 845 1954

15 Graves, W P & Van Smith G Cirsoid neurysm of the uterus Am J Obstet Gynecol 14 30 1927

16. Hibba d B M Pryor Jones, D & Scarrow G D Cirsoid neurysm of the uteru as cause of menorrhagia J Obstet Gynecol Br Commonw 79 855 1972

17 Hoge R H Arteriovenous fist la of the terus South Med J 48 18, 1955

18 Howard, L R Iatrogenic arteriovenous al s of a terine artery nd vein Report of c se Obstet Gynecol 31 255 1968

19 Leibold, R W Keefer F J & Curry J L. Surgical excision of pelvic fistula following successful

treatment of chorionepithelioma. Obstet Gynecol *31* 337 1968

20. Liggon, G. C.: Uterine arteriovenous fistula. Report of case. Obstet Gynecol *23* 214 1964
21. Morley G. W & Lindenauer S. M.: Arteriovenous fistula following pelvic operations. Obstet Gynecol *31* 622, 1968
22. Van Ostrand, J. R., Wellenbeck, B. & Forsberg, C.: Vascular lesions of the female pelvis. Am J Obstet Gynecol *85* 966, 1963.
23. Reynolds, R. P. Owen, C. J & Cantor M. O. Arteriovenous aneurysm of uterine artery and [illegible]. JAMA *141* 841 1949
24. Wideman, G. L., Greenlee L. C. & Jones, W. N. Arteriovenous aneurysm of the uterine artery and [illegible] following total abdominal hysterectomy. Am J Obstet Gynecol *78*. 200, 1959
25. Williams, G. A.: Arteriovenous aneurysm of the uterus. Am J Obstet Gynecol 67 198 1954

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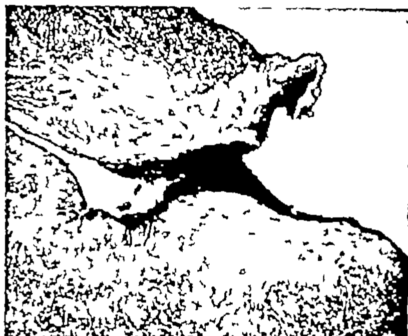


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1 Benson, R C Dotter C I Peterson, C G Metcalf J Bristow J D & Krausbaar O F Congenital arteriovenous fistula and pregnancy Am J Obstet Gynecol 9 672, 1965

2 Berlin, A C & Horwitz, D Arteriectasis of uterine artery Am J Obstet Gynecol 64 347 1952

3 Borell, U & Fernström I Arteriovenous fistulae of the uterus and adnexa Acta Radiol 49 1 1949

4 — Ectopic pregnancy associated with arteriovenous fistula. Acta Obstet Gynecol Scand 38 404 1959

5 Borell, U Fernström, I Moberger G & Olsson, L The Diagnosis of Hydatidiform Mole, Malignant Hydatidiform Mole and Choriocarcinoma with Special Reference to the Diagnostic Value of Pelvic Arteriography Thomas, Springfield, Ill., 1966.

6 Camp, O B. Arteriovenous fistula following hysterectomy Am J Surg 86 40 1953

7 Cockshott W P & de V Hendrickse, J P Persistent arteriovenous fistulae following chemotherapy of malignant trophoblastic disease Radiology 88 329 1967

8 Decker D G Fish, C & Juergens, J Arteriovenous fistulas of the female pelvis. A diagnostic problem Obstet Gynecol 31 799 1968

9 Dubreuil, G. & Loubat, E. Anévrysme cirsoïde de l'utérus Ann Anat Pathol 3 697 1926

10 Fernström, I Personal communication.

11 Fontaine, R Le Gal, Y Kieny R Bollack, C L & Chaddad, F Cirsoid aneurysm within the framework of arteriovenous fistulae A propos of 5 personal cases. Lyon Chir 63 33., 1967

12. Frencken, V A M & Landman, G H M Cirsoid aneurysm of the uterus: Specific arteriographic diagnosis. Report of case Am J Roentgenol 95 755, 1965

13 Gaines, J & Greenwald, J C. Uterine arteriovenous fistula Am J Obstet Gynecol 65 997 1953

14 Gardner H J Cirsoid aneurysm of the uterus Am J Obstet Gynecol 68 845 1954

15 Graves, W P & Van Smith, G Cirsoid aneurysm of the uterus. Am J Obstet Gynecol 14 30 1927

16 Hibbard, B M Prysor Jones, D & Scarrow G D Cirsoid aneurysm of the uterus a cause of menorrhagia J Obstet Gynaecol B Commonw 79 [illegible] 1972

17 Hoge R H Arteriovenous fistula of the uterus South Med J 48 18 1955

18 Howard L R Iatrogenic arteriovenous sinus of a uterine artery and vein Report of case Obstet Gynecol 31 _55 1968

19 Leibold, R W Keefer F J & Curry J L Surgical excision of pelvic a-v fistula following successful

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PLETHYSMOGRAPHIC STUDIES OF VENOUS VOLUME IN THE LOWER LEGS DURING NORMAL PRIMIPREGNANCY

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Abstract. The venous volume of the lower legs was examined plethysmographically in 60 primigravidae, grouped according to duration of pregnancy. The three periods were 9–13 weeks (Group A), 15–22 weeks (Group B) and 36–41 weeks (Group C). In addition to the examination during pregnancy in Group A examinations were performed 1 day and 2 weeks after abortion, while in Groups B and C the examinations were made 2 weeks after abortion and delivery respectively. The recordings were made at venous occlusion pressures of 40, 60 and 80 mmHg. Contrary to the information given in the literature on gravidae, it has not been possible to detect any difference in the venous volume of the lower legs during primipregnancy.

Some authors consider the appearance of superficial veins on, for instance, the legs to be a feature of pregnancy (12, 22, 23, 26). On the assumption that pregnancy has a tonus-lowering effect on the smooth muscles of the veins, an increase in the progesterone level, as during pregnancy, has been cited as an aetiological factor in the development of varices (22). Another aetiological factor seems to be the increased venous pressure in the legs during the latter half of pregnancy (see 24). The incidence of varicose veins during pregnancy is variously quoted between 11 and 40% in series including both primi- and multigravidae (26, 31). Increased distensibility of the veins during pregnancy has been demonstrated plethysmographically in the fingertips (23) and in the calves and forearms (13). Intravenous administration of estradiol 17 β to nonpregnant women also gave an increased venous volume (14). The distensibility of the veins increases by 150% during pregnancy but returns to normal 8–12 weeks after delivery (18). This distensibility was considered to be even more pronounced when varices are present (18). Clinical observations have verified that more than one-third of the varices that arise during pregnancy do so during the first trimester (26). None of the investigations reported have been based solely on groups of primigravidae.

Using ascending phlebography as described by Greitz (15), it has not been possible to detect any change in the venous diameter of the large veins of the leg during the first half of pregnancy (28). Nor has it been possible during pregnancy to detect any increase in the distensibility of the veins in a segment of the forearm examined with the aid of volume plethysmography combined with direct measurements of the venous pressure; unaltered values have also been recorded 2 weeks and approximately 16 weeks after delivery (10). Both of the above examinations were performed on primigravidae. There is reason, then, to question whether changes in the venous volume appear during the first pregnancy. In order to elucidate this problem, uniform, distinctly defined groups of primigravidae were plethysmographically examined at different stages of pregnancy.

MATERIAL

The subjects were 60 healthy primigravidae, constituting three separate series defined in an earlier study and designated Groups A, B and C (29). The number of subjects examined in Groups A and C was 22, and in Group B 16. Details of the groups are given in Table I.

METHODS

Venous occlusion plethysmography (VOP)

According to Wood (33) and other investigators, the waterfilled occlusion plethysmograph is the best apparatus

BOOKS RECEIVED

Handbuch der experimentellen Pharmakologie Heffter Heubner New Series, Volume 35 Part I, Voss, H. E. and Oertel, G *Androgene I* 686 p. Price DM 298 — US$ 110.30 Springer V rlag, 1973

The first of two planned v lumes concerning history chemistry physiology patho-physiology and pharmacology of androgens has been published. The book has all the good qualities of a German manual and is recommended to research workers in reproductive physiology and endocrinology

Urethritis non gonorrhoica des Mannes, by J Söltz-Szöts, 83 p. Springer Verlag, Price DM 19.80, US$ 7.00.

A small olume containing what a gynecologist ought to know about the non-specific urethritis in the male and its role concerning infertility

Gertrude von B genen and Miriam E Simpson Post natal development of the ovary in homo sapiens and macaca mulatta and induction of ovulation in the ma caque. Yale University Press, London, 1973 305 pages. Price £11 75

A most beautiful collection of plates concerning the postnatal development of the ovary in the human and the macaque. The book is completed with a study concerning the response to different gonadotropins It is recommended to scientists working in the field of reproduction.

D V I F lrweather and T K A B Eskes Amniotic Fluid Research and clinical application. Excerpta Medica, Amsterdam 1973 360 pages Price Dfl 91 00 (ca $28.50)

This book is the most complete text book concerning clinical and scientific aspects on the amniotic fluid in women. It is recommended to all obstetricians and gynaecologists, who use cytological, chemical or physical studies on the amniotic fluid for the examination of the health and maturity of the fetus.

Handbuch der speziellen pathologischen Anatomie und Histologie Volume VII Part 4 *Weibliche Geschlechtsorgane Vulva V gina, Urethra.* Springer Verlag, Berlin, Heidelberg and New York, 1972, 914 pages, 378 illustr Price DM 396 —

Handbook by Henke Lubarsch which has been brought up to date by well known specialists in the field of gynaecological pathology The very complete chapter concerning dysplasia-carcinoma in situ-microcarcinoma cer vicis uteri, containing new definitions and terminology has also been included. The book ought to be included in the library of all university departments of obstetrics and gynaecology

The Prenatal Diagnosis of Hereditary Disorders by Aubrey M lunsky Charles C. Thomas, Publisher Springf ld, Illinois, USA, 1973 254 p. Price $11 75

Th list of hereditary or sex-linked disorders in hich amniocentesis and chromosomal examination is indicated ncreases every year Ultrasound studies, fetoscopy and other methods ha e offered new possibilities to mak an antenatal diagnosis of malformations Milunsky book gives an excellent review of the subject The bibliography contains 891 references. The book is recommended to everybody interested in obstetrics and perinatology

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ANNOUNCEMENTS

The VIth Asian Congress of Obstetrics and Gynaecology will take place in Kuala Lumpur Malaysia, 20– 7 July 1974

Information. The Organising Secretary VIth Asian Congress of Obstetrics and Gynaecology Department f Obstetrics, Univ rsity Hospital, Kuala Lumpu M laysia.

The 4th European Congress f Perinatal Medicine takes place in Prague Czechoslovakia, n A gust 28–31 1974 Congress Secret riat Czechoslo ak Medical Society J E. Purkyne 4th European Congress f Perinatal Medicine Sokolská 31 120-26 Praha 2, Czechoslov kia.

The IVth International Congress on H rmonal Steroid will be h ld in Mexic City September 7 1974 Congress Secretariat. D J L. Mateos, Apartado Postal 73-13., Mexico 73 D.F M xico.

The 25th Congress of La Fédération des Sociétés de Gynécologie et d'Obstétrique de Langue Française w ll t k place in Mo t al C nada, n 24-28 September 1974

F further nformation nte Congress Bure u, 2065 rue Alexandre D sève Montreal 133 Prov nce Quebec, Canada

The XI International Cancer Congress ll be held in Florence, October 1974 F rther nf rmation Secretariat f th XIth International Cancer Congress, Istituto Nazionale T mori, V a G. Venezi 1 20133 Milano, Italy



Fig. 1. Venous volume—and venous emptying curve in primigravida. The marked drop in the curve is due to withdrawal of water from the plethysmograph system. On release of the occlusion pressure (80 mmHg) the volume of the limb decreases *rapidly*.

period following abortion. The analyses were performed as double determinations by Klopper's method (17). The error of method was 10% (6).

The haemoglobin determinations were performed by method described in an earlier paper (27).

RESULTS

A typical venous volume—and venous emptying curve is shown in Fig. 1. The venous volume was calculated both with and without a correction for the estimated net filtration, and since no significant differences were noted, only the uncorrected values are indicated.

The magnitude of the filtration rate was calculated to be 0.13 ml/min/100 ml tissue at a venous occlusion pressure of 60 mmHg and 0.24 ml/min/100 ml tissue at 80 mmHg. These values are the same for Groups A, B and C. The rate of filtration for a given pressure has therefore been regarded as constant during pregnancy.

There are no differences in the venous volumes of the right and left lower leg in Groups B and C. In Group A the venous volume is greater in the left leg than in the right at venous occlusion pressure of 60 mmHg and 80 mmHg. The individual mean value for both legs was used in the statistical analysis. The venous volumes at the three venous occlusion pressures used are unaltered during primipregnancy and no changes occurred after abortion or delivery (Table II).

Venous emptying rate (8) was equal in all groups and in no case was there reason to suspect venous thrombosis. These findings are still under investigation (2).

In Group A the venous volumes were also recorded 24 hours after abortion. There are no significant differences between these values and those recorded during pregnancy and 2 weeks after abortion. The pregnandiol level fell from a mean value of 4.1 mg ± 2.8 mg/24 hours' urine to 1.4 mg ± 1.0 mg/24 hours one day after abortion. During the same period the haemoglobin values diminished from 11.3 g% ± 0.7 g% to 11.0 g% ± 0.7 g%.

DISCUSSION

The present investigation provides no evidence of a general increase in the venous volume (at the lateral pressure used) during primipregnancy. The results therefore agree with conditions observed by Duncan & Bernard (10) in the forearms when the venous pressure was recorded intravascularly in connection with volumetric plethysmographic measurements. Like the present study this investigation was performed on primigravidae, which is not true of series in other reports. The increased distensibility demonstrated in the finger tip veins (23) becomes less certain when one considers the great abundance of arteriovenous anastomosis in this region. Goodrich & Wood (13) based their conclusions on a slight difference in the values for forearm segments and segments of the lower legs during the final phase of pregnancy

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[1] The analyses were performed at the Hormonal Laboratory of the Sabbatsberg Hospital, Karolinska Institutet, Stockholm (Professor Mirjam Furuhjelm).

Table I Age menarche duration of pregnancy and haemoglobin concentration for Groups A B and C

Mean ± S.D Ranges in brackets

	Group A (n = 22)	Group B (n = 16)	Group C (= 22)
Age, years	18.8 ± 3.3 (15–27)	19 6 ± 4.4 (15–32)	23 4 ± 3.4 (19–30)
Menarche, years	13 1 ± 1 1 (11 15)	13 1 ± 1 1 (11 15)	—
Duration of pregnancy weeks	12.0 ± 1.2 (9–13)	17.3 ± 1.9 (15–22)	38.0 ± 1 0 (36–41)
Haemoglobin, g% pregnancy	11.3 ± 0.7 (10 4–12.9)	10.8 ± 0.9 (9.3–12.2)	11 6 ± 0.6 (10.5–13.2)
Haemoglobin, g%, after pregnancy	12.1 ± 0.8 (10.6–13 4)	11 6 ± 0 7 (10.3–12.4)	12.9 ± 0 8 (11.2–15.2)

for measuring venous distensibility In the examinations described below the apparatus was of the type described in detail by Dahn (7) and used by for instance, Eiriksson & Dahn (11) in studies of venous volumes and venous emptying rates in nonpregnant patients with and without varices of the legs.

Theory

A segment of the lower leg enclosed in a plethysmograph will increase in volume when a venous occlusion cuff is applied proximally Afte application of the femoral occlusion cuff a rapid initial rise is recorded on the connected recording device as a sign of increased venous congestion. This initial steep portion of the curve levels off rather quickly when a state of equilibrium is established between the applied pressure and the tension of the veins (9). If stasis is maintained, the venous blood begins t pass the femoral occlusion cuff and the curve then rises t only a negligible extent, probably as an indication of the capillary net filtration. The magnitude of this net filtration at different occlusion pressures is difficult to determine more exactly since the pressure exerted in the capillaries can only be calculated appro imately However it is now generally assumed that under conditions of rest 80% of the applied occlusion venous pressure is reverted to the capillary level (25). In this condition the increased venous pressure can disturb the balance between the hydrostatic and colloid osmotic pressures in the capillaries and cause an ncrease in the net filtration. There is linear relationship between the rate of filtration and the pplied venous pressure (19). It has been demonstrated that the net filtration in the forearm generally remains constant during pregnancy and is of the same magnitude a during nonpregnancy (32). At a venou occlusion pressure of 40 mmHg Spetz observed a net filtration of 0.10 ml/ml 100 ml tissue and 0.19 ml/min 100 ml tissue at a pressure of 60 mmHg. However these values are higher than those calculated for the lower legs during nonpregnancy (9). Drury & Jones reported a value of 0.04 ml min × 100 ml tissue at a venous occlusion pressure of 40 mmHg and 0 07 ml min × 100 ml tissue at a pressure of 60 mmHg.

In ascertaining the venous volume not only the volume of blood in the veins is determined, but also the portion of fluid that has been filtered as a result of venous stasis and the increased hydrostatic pressure in the capillaries (net filtration). The venous volume at a specific occlusion pressure (40, 60 or 80 mmHg) is expressed in ml/100 ml of tissue and indicates the increase in volume noted when a steady level has been indicated on the recorder. The increase in volume in the congested segment is, however mainly due to distension of the veins not only of the larger veins but also of the small veins not subject to examination by phlebography (9 20). Owing to the pressure exerted by the water in the plethysmograph and the pressure drop during transmission from the cuff to the deeper parts of the limbs, the lateral venous pressure varied up to about 55 mmHg. This range is low compared with the venous pressure observed in the upright position, but is higher than the lateral pressure used in investigations of the venous volume during pregnancy published earlier

Procedure

The experimental conditions were mainly the same as reported earlier (29) and the examinations were performed in connection with the blood flow determinations (29). The patients were in the supine position with both lower legs placed in plethysmographs at midthoracic height i.e. at the level of the right auricle The pressure exerted by the water in the plethysmograph on the middle of the lower leg segment (approximately 10 mmHg) was considered sufficient t empty the veins before each new recording was made (11). Proximal occlusion cuff at varying pressures (40, 60 and 80 mmHg) were applied to the th ghs just above the knee caps. N distal cuffs were used. The cuff were inflated and the changes in volume thereby produced were recorded on a kymograph. When a teady level was reached at each venous pressure applied, the cuffs were deflated and the veins emptied (8). On each occasion time was llowed for a return to a state of equilibrium before pplying the next occlusion pressure

I calculating capillary f ltration, net filtration was considered to be so slight at cuff pressures up to 40 mmHg that it has been neglected. Abov this occlusion pressure the net filtration was considered to be linear The slope of the volume curv after the f rst rapid rise was regarded as an ndication f the net filtration.

The error of single measurement was calculated from n double determinations performed on two consecutive days. In determinations of the venous volume this error was found to be 15–30% (the higher value applying to a venous occlusion pressure of 40 mmHg). Reproducibility was poorer in determinations of the capillary filtration, and the error of a single measurement was 30–50%.

Det rmination of Pregnandiol

Twenty-four-hour quantities of urine were collected during early pregnancy (8–13 weeks) nd during the 4-hour

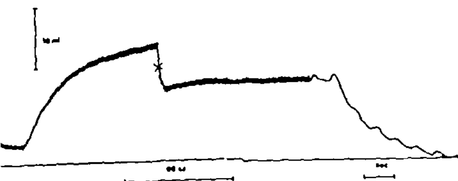


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Table II *Venous volume (ml/100 ml tissue) at different durations of pregnancy and at different venous occlusion pressures*

pr = pregnancy apr = 2 weeks after abortion or delivery
Mean ± S.D RL = right leg, LL = left leg, Diff = pr − apr

	Venous volume, ml/100 ml tissue								
	40 mmHg			60 mmHg			80 mmHg		
	pr	apr	diff	pr	apr	diff	pr	apr	diff.
Group A	*n* = 22	*n* = 22		*n* = 22	*n* = 22		*n* = 22	*n* = 22	
RL	1.5±0.8	1.6±0.9		2.7±1.0	2.8±1.0		3.7±1.1	3.8±1.1	
LL	1.8±0.9	1.7±1.1		3.2±1.1	3.1±1.2		4.2±1.1	4.1±1.3	
Mean RL and LL	1.7±0.8	1.7±0.9	+0.1	3.0±1.0	2.9±1.0	+0.1	4.0±1.0	3.9±1.1	+0.1
Diff RL and LL	−0.2	−0.2		−0.5	−0.2		−0.5	−0.2	
Group B	*n* = 16	*n* = 16		*n* = 16	*n* = 16		*n* = 16	*n* = 16	
RL	1.6±1.0	1.3±0.9		3.1±1.4	2.5±1.3		3.9±1.4	3.4±1.3	
LL	1.4±0.7	1.4±0.6		2.7±0.9	2.6±0.7		3.5±1.0	3.5±0.7	
Mean RL and LL	1.5±0.6	1.4±0.7	+0.2	2.9±1.0	2.6±0.9	+0.3	3.8±1.0	3.5±0.9	+0.3
Diff RL and LL	+0.3	0.0		+0.5	−0.1		+0.4	−0.1	
Group C	*n* = 22	*n* = 20		*n* = 22	*n* = 20		*n* = 22	*n* = 20	
RL	1.7±0.8	1.7±1.1		3.0±0.9	2.8±1.2		3.7±1.0	3.6±1.1	
LL	1.6±0.9	1.8±0.9		2.9±1.1	3.1±1.1		3.9±1.1	3.9±1.1	
Mean RL and LL	1.7±0.7	1.8±0.7	−0.1	3.0±0.9	3.0±1.1	+0.0	3.8±0.9	3.8±1.1	0.0
Diff RL and LL	+0.2	−0.1		+0.1	−0.2		−0.1	−0.3	
Differences between groups (mean RL and LL)									
A − B	*+0.2*	*+0.3*		*+0.1*	+0.3		+0.2	+0.4	
A − C	0.0	−0.1		0.0	−0.1		+0.2	+0.1	
B − C	−0.2	−0.4		−0.1	−0.4		0.0	−0.3	

($0.01 < P < 0.05$), ($0.001 < P < 0.01$).

as compared with values recorded a few weeks after delivery In addition only pressures of up to 30 mmHg were used. It is therefore probable that the maximum distensibility of the veins was not measured in the two examinations.

The present investigations were performed with lateral venous pressure up to about 55 mmHg which is equal to or higher than the ranges used earlier However the orthostatic venous pressure of the legs is higher than the pressures used in the present study It cannot be ruled out that differences in venous volume during pregnancy could exist. The main reason why a higher lateral pressure was not used in this investigation was the probable effect on the arterial inflow to the leg. In the standing position when lateral venous pressure is considerably higher than during the plethysmographic studies, phlebographic investigations of the diameter of the veins of the legs have also failed to produce evidence of dilatation of the large leg veins during the first half of pregnancy (28).

The time for the examination following abortion or delivery was chosen after investigation of five women in Group A had revealed that the venous volume 2 weeks after early abortion was the same as that determined 8 weeks following abortion (mean difference 0.1 ± 0.4 ml/100 ml tissue at an occlusion pressure of 60 mmHg). Thus the venous volumes measured 2 weeks after early abortion in Group A were considered to be approximately representative of nonpregnancy Such an investigation was not performed in Groups B and C and in these groups the post pregnant values may not represent the non-pregnant state (29).

The pregnandiol excretion during the first trimester is of a magnitude (4.1 mg/24 hours urine) that can be seen during normal secretory phase It seems hardly likely that a progesterone level corresponding to the above excretion would influence the distensibility of healthy veins but the exposure time may perhaps have been too short.

An increase in the venous pressure in the leg veins during pregnancy has been demonstrated by different investigators (3 4 5 24 27) in which

respect unanimity prevails, but this is not the case when it comes to explaining the cause of the pressure rise. It is possible that the increase in pressure to which the veins of the leg are subjected at least during the latter part of pregnancy and which is probably the result of uterine pressure on veins leading from the legs and pelvis (24 30) as well as of the dynamic function of the placenta (1 30) results in deterioration of the eins in women with this predisposition. In a subsequent pregnancy varices may appear owing to additional pressure and hormonal effects on those deteriorated veins.

The difference in the venous volume of the right and left leg in Group A at occlusion pressures of 60 and 80 mmHg is rather difficult to interpret. Lateral differences in the course of the iliac eins and their relation to the corresponding arteries (21) as well as the mainly sinistral localization of the sigmoid colon, have been suggested as possible causes of the higher incidence of arices of the left leg Dilatation of arteriovenous shunts in the calf regions—demonstrated by Haeger (16) and perhaps more frequent in the left leg—could cause the lateral difference during early pregnancy This difference can neither be detected during later pregnancy—Groups B and C nor after pregnancy

REFERENCES

1 Bickers, W Sth Med J 35 593, 1942.
2 Bjerle, P & Sandström, B T be published.
3 Born E C S Amer J Med Sci 195 1, 1938.
4 — Arch Intern Med 11 1305 1938.
Barwell, C S Strayhorn, W D Flickinger, D Corlette M B Bowerman, E P & Kennedy J A Arch Intern Med 6, 979 1938
6 Carlström, K & Furuhjelm, Mirjam Personal communication, 1969
7 Dahn, I Acta Chir Scand 130: 42, 1965
8 Dahn, I & Eriksson, E Acta Chir Scand, Suppl. 399 33 1968
9 Drury A N & Jones, N. W Heart *14* 55 1927
10 Duncan, L B, Slavin & Bernard, A J Obstet Gynaecol Br Comm *75* 142, 1968.
11 Eriksson, E & Dahn, L Acta Chir Scand, Suppl. 398. 19 1968.
12. Foote, R R. Varicose Veins, 2nd ed. London, 1954
13 Goodrich, S. M. & Wood, J E: Amer J Obstet Gynecol 90 740, 1964.
14. — Amer J Obstet Gynecol 96 407 1966.
15. Greitz, T Acta Radiol (Stockh) *42*. 421 1954.
16 Haeger K., Varicose veins. *In* Venous and Lymphatic Disorders of the Leg (ed. K. Haeger), chap. IV p. 43. Scandinavian University Books, Lund, 1966.
17. Klopper A., Michie, Eileen A. & Brown, J B J Endocrinol *12*. 209 1955
18. Kramer M., Damerow, L. & Klink, G Handbuch der experimentellen Pharmakologie, Band XXII, 507 1968.
19 Landis, E. M. & Gibbon, J H., J Clin Invest *12* 105, 1933.
20. Litter J & Wood, J E. J Clin Invest *33*. 789 1954.
21 Ludwig, H Fortschritte der Medizin *17* 693, 1965
22 McCausland, A. M. West J Surg *51* 199 1943.
23 McCausland, A. M., Hyman, C., Winsor, T & Trotter A. D Amer J Obstet Gynecol *81* 472, 1961.
24. McLennon, C. E.: Amer J Obstet Gynecol *45* 568, 1943.
25 Mellander S Acta Physiol Scand, Suppl. 50. 176, 1960.
26. Mullane, J Amer J Obstet Gynecol *63* 620, 1952.
27 Runge, H Arch Gynäk *122*: 142, 1924.
28. Sandström, B & Lövegren, L Acta Obstet Gynecol Scand *49* 375 1970.
29 Sandström, B Acta Obstet Gynecol Scand 1972 a. *53* 1 1974
30. Scott, D B. & Kerr M. G. J Obstet Gynaecol Br Comm 70. 1044 1963.
31 Siegler J Am J Surg *44* 403 1939
32. Spetz, S Acta Obstet Gynecol Scand *44* 227 1965
33 Wood, J E. The veins normal and abnormal function Churchill, London, 1965

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Mean ± S.D RL = right leg, LL = left leg, Diff = pr − apr

	Venous volume, ml/100 ml tissue								
	40 mmHg			60 mmHg			80 mmHg		
	pr	apr	diff	pr	apr	diff	pr	apr	diff
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RL	1.5±0.8	1.6±0.9		2.7±1.0	2.8±1.0		3.7±1.1	3.8±1.1	
LL	1.8±0.9	1.7±1.1		3.2±1.1	3.1±1.2		4.2±1.1	4.1±1.3	
Mean RL and LL	1.7±0.8	1.7±0.9	+0.1	3.0±1.0	2.9±1.0	+0.1	4.0±1.0	3.9±1.1	+0.1
Diff RL and LL	−0.2	−0.2		−0.5	−0.2		−0.5	−0.2	
Group B	n = 16	n = 16		n = 16	n = 16		n = 16	n = 16	
RL	1.6±1.0	1.3±0.9		3.1±1.4	2.5±1.3		3.9±1.4	3.4±1.5	
LL	1.4±0.7	1.4±0.6		2.7±0.9	2.6±0.7		3.5±1.0	3.5±0.7	
Mean RL and LL	1.5±0.6	1.4±0.7	+0.2	2.9±1.0	2.6±0.9	+0.3	3.8±1.0	3.5±0.9	+0.3
Diff RL and LL	+0.3	0.0		+0.5	−0.1		+0.4	−0.1	
Group C	n = 22	n = 20		n = 22	n = 20		n = 22	n = 20	
RL	1.7±0.8	1.7±1.1		3.0±0.9	2.8±1.2		3.7±1.0	3.6±1.1	
LL	1.6±0.9	1.8±0.9		2.9±1.1	3.1±1.1		3.9±1.1	3.9±1.1	
Mean RL and LL	1.7±0.7	1.8±0.7	−0.1	3.0±0.9	3.0±1.1	+0.0	3.8±0.9	3.8±1.1	0.0
Diff RL and LL	+0.2	−0.1		+0.1	−0.2		−0.1	−0.3	
Differences between groups (mean RL and LL)									
A − B	+0.2	+0.3		+0.1	+0.3		+0.2	+0.4	
A − C	0.0	−0.1		0.0	−0.1		+0.2	+0.1	
B − C	−0.2	−0.4		−0.1	−0.4		0.0	−0.3	

(0.01 < P ≤ 0.05), (0.001 < P ≤ 0.01).

as compared with values recorded a few weeks after delivery. In addition, only pressures of up to 30 mmHg were used. It is therefore probable that the maximum distensibility of the veins was not measured in the two examinations.

The present investigations were performed with lateral venous pressure up to about 55 mmHg which is equal to or higher than the ranges used earlier. However, the orthostatic venous pressure of the legs is higher than the pressures used in the present study. It cannot be ruled out that differences in venous volume during pregnancy could exist. The main reason why a higher lateral pressure was not used in this investigation was the probable effect on the arterial inflow to the leg. In the standing position, when lateral venous pressure is considerably higher than during the plethysmographic studies, phlebographic investigations of the diameter of the veins of the legs have also failed to produce evidence of dilatation of the large leg veins during the first half of pregnancy (28).

The time for the examination following abortion or delivery was chosen after investigation of five women in Group A had revealed that the venous volume 2 weeks after early abortion was the same as that determined 8 weeks following abortion (mean difference 0.1 ± 0.4 ml/100 ml tissue at an occlusion pressure of 60 mmHg). Thus the venous volumes measured 2 weeks after early abortion in Group A were considered to be approximately representative of nonpregnancy. Such an investigation was not performed in Groups B and C and in these groups the post pregnant values may not represent the nonpregnant state (29).

The pregnandiol excretion during the first trimester is of a magnitude (4.1 mg/24 hours urine) that can be seen during normal secretory phase. It seems hardly likely that a progesterone level corresponding to the above excretion would influence the distensibility of healthy veins but the exposure time may perhaps have been too short.

An increase in the venous pressure in the leg veins during pregnancy has been demonstrated by different investigators (3, 4, 5, 24, 27), in which

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THE EFFECT OF OXYTOCIN ON HUMAN UTERINE BLOOD FLOW MEASURED BY LOCAL HYDROGEN CLEARANCE

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Abstract. Local myometrial and cervical uterine blood flow were measured repeatedly in 45 women by the hydrogen gas clearance method. Mean myometrial blood flow in the postmenopausal women was 57.5 ± 6.07 ml/min 100 g and in the women of fertile age 105.5 ± 8.36 ml/min 100 g. When oxytocin was given intravenously between measurements, no effect on myometrial blood flow was obtained in the postmenopausal women. In women of fertile age, myometrial blood flow was reduced to 18.9 ± [illegible]% of controls in the proliferative phase and to 33.1 ± 7.62% in the secretory phase 2 minutes after injection of oxytocin, whereas cervical blood flow in both phases showed no or only a slight reduction.

Blood flow in the uterine artery measured with electromagnetic flowmeter in anaesthetized non pregnant women (9) was markedly reduced after intravenous injection of oxytocin. By heat conductivity as a relative measure of local myometrial blood flow Prill (11) found a flow decrease of 20–50% after injection of oxytocin, but no information was given about the age and menstrual phase of the patients.

The purpose of the present investigation was to study the effect of oxytocin on local myometrial and cervical uterine blood flow in women in the proliferative and secretory phase of the menstrual cycle and in the postmenopausal period.

METHODS

Local blood flow was measured by the hydrogen gas clearance method (1). The technique for measurement of cervical (7) and myometrial (8) blood flow is previously described. The insertion of the electrodes was performed with the patient lying in lithotomy position, and no anesthesia or other drugs were given.

The electrodes in the cervical tissue were inserted before the instrument for simultaneous myometrial flow measurement was introduced through the cervical canal. The instrument for recording myometrial blood flow allows simultaneous measurements in two different areas, and two electrodes were usually placed in the cervical tissue. Two subsequent measurements were performed in the same patient with the electrodes in the same position. The time between insertion of electrodes and first measurement was 20–30 min and between onset of the first and second measurement 20–30 min.

Oxytocin was given as Pitocin (Parke, Davis & Company) into an antebrachial vein. The injection of oxytocin, 5 IU lasted 1 1/2–3 min, and the second hydrogen desaturation period started 1 1/2–2 min after the injection was finished.

The delay between removal of the hydrogen gas added to the respiratory air and onset of hydrogen desaturation was the same in the first and second measurement. Hydrogen desaturation curves recorded after the injection of oxytocin often showed an increasing washout rate. Semi-logarithmic plots of these curves therefore were curved with the convexity upwards. Blood flow was estimated from the disappearance rate obtained from the tangents to the plotted curve at 2, 4, 6, 8 and 10 min after onset of washout. The onset of desaturation was found by considering the first and second measurement to have the same delay before desaturation started. The mean flow from the two electrode sites in each patient was first calculated. Then mean values of the groups were calculated from the mean for each patient. The blood flow differences between groups were tested by the Wilcoxon rank test and the Student' t-test. The mean values are given ± S.E.

MATERIAL

Repeated uterine blood flow measurements were performed in 45 women, 17 postmenopausal (the youngest 53 years old) and 28 of fertile age (the oldest 45 years). T[illegible] of them were studied both in the proliferative and the secretory phase, indicated in the tables with [illegible]

The material consisted of the four different groups:

1. Controls (Table I): 12 patients
2. Proliferative phase (Table II): 10 patients

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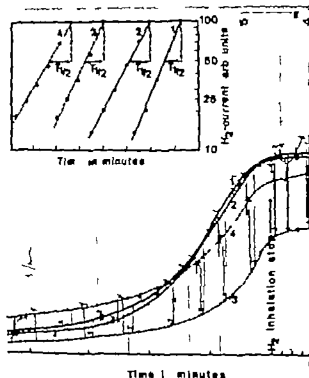


Fig. 1. Hydrogen desaturation curves before (upper part) and after (lower part) oxytocin was given to patient 28, Table III. The unbroken lines from the myometrium, the dotted lines from the cervix. Insets: Semi-logarithmic plots of the desaturation curves. Straight lines show the slopes used for flow calculation. Two and six minutes after injection of oxytocin (inset, lower part) myometrial flow is calculated as the tangents to the slightly upwards convex curves. Blood flow before oxytocin was given (upper part) was 102.7 and 79.2 ml/min 100 g respectively in the myometrium and 89.4 and 69.0 ml/min 100 g respectively in the cervix. The myometrial flow calculated from the tangents to the plots at two and six minutes after injection of oxytocin was 20.4 and 25.7 ml/min 100 g and 16.9 and 22.0 ml/min 100 g respectively. The cervical flow was 63.0 and 60.3 ml/min 100 g respectively.

Table I *Repeated myometrial blood flow measurements without drugs (control group)*

Pat. no	Age (y)	Diagnosis	First measurement Ml/min 100 g Each electrode	Mean	Second measurement Ml/min 100 g Each electrode	Mean	In percent of flow in the first measurement Each electrode	Mean
1	53	Urinary incontinence	29.5 39 6	34 6	23.1 34 7	28.9	78.3 87.6	83.0
2	60	Suburethral cyst	25.7 36.5	31 1	23 9 30.1	27.0	93.0 82.5	87.8
3	50	Vaginal prolapse and uterine myoma	19.3 25 7	22.5	19.3 25 7	22.5	100.0 100.0	100.0
4	59	Vaginal prolapse	63 0 43.3	53.2	49.5 33 0	41.3	78.6 76.2	77 4
5	67	Vaginal prolapse	21 0	21 0	18.5	18.5	88.1	88.1
6	63	Uterine prolapse	40 8 23.9	32.4	40.8 23.9	32.4	100.0 100.0	100 0
7	54	Endometrial carcinoma treated with radiation	77 0 77 0	77.0	74 9 69.3	72.1	97.3 90 0	91 7
8	26	Infertility	77 0	77 0	60 3	60.3	78.3	78.3
9	29	Metrorrhagia	44 7 57.8	51.3	40.8 57.8	49.3	91.3 100.0	95.7
10	33	Urinary incontinence	218.6 193 6	206.1	177.2 169 9	173 6	81 1 87.8	84.5
11	28	Perineal laceration	154.0	154.0	173.3	173.3	112.5	112.5
12	32	Infertility	96 8 96.8	96.8	81.5 77 0	79.3	84 2 79.5	81 9
Mean ±S.E.								90.2 ±3.0.

3 Secretory phase (Table III) 13 patients
4 Postmenopausal period (Table IV) 10 patients

In the control group (Table I) myometrial blood flow *was measured repeatedly* in 12 women, 7 postmenopausal and 5 in fertile age, without giving drugs between the first and second measurement. The age and the diagnoses, the disease or gynaecological complaints of the women are shown in Table I. By pelvic examination and by measurement of the uterine cavity the uterus was within normal size in all women.

In 23 women with regular menstrual cycles, uterine blood flow was recorded before and immediately after injection of oxytocin. Ten of the women were in the proliferative phase (Table II) and 13 in the secretory phase (Table III). The age of the women, the disease or complaints and day after onset f the last period are collected in Tables II and III. Phase decision was confirmed by vaginal smears and endometrial biopsies. Vaginal smears were taken within 24 hours before, and endometrial biopsies taken within 36 hours after the measurements. Except I women with small uterine myomata, the uteri were within normal size

Myometrial blood *flow measurements were performed* in 10 postmenopausal women before and fter injection f oxytocin (Table IV). The women were 53 years ld or more and they were all suffering from vaginal or/and uterine prolapse. The ut rus was found to be normal in size in all of them.

RESULTS

Mean myometrial blood flow by the first measurement in the 17 postmenopausal women was $57\ 5 \pm 6.67$ ml/min 100 g which was significantly different ($p = 0\ 01$) from the mean flow on $105\ 5 \pm 8\ 36$ ml/min 100 g in the 28 women of fertile age

In the control group (Table I) blood flow obtained by the second measurement averaged $90\ 2 \pm 3\ 02$ % of flow in the first measurement.

Myometrial blood flow measurements in 10 women in the proliferative (Table II) and in 13 women in the secretory (Table III) phase showed in most cases desaturation curves with different shape before and after injection of oxytocin.

Fig. 1 demonstrates hydrogen desaturation curves before (upper part) and after (lower part) oxytocin was given to patient number .8 in Table III The unbroken lines show myometrial, the dotted lines cervical desaturation. An initial delay before desaturation of about 60 sec is taken into consideration both before and after injection of oxytocin The inset demonstrates the semi-logarithmic

Table II. *Effect of oxytocin on uterine blood flow proliferative phase*

Pat. no	Age (y)	Diagnosis	Day after last menstr period	Myometrial blood flow: Before oxytocin, Ml/min 100 g, Each electrode	Mean	Two minutes after oxytocin, Ml/min 100 g, Each electrode	Mean	In percent of the flow before oxytocin, Each electrode	Mean	Cervical blood flow: Before oxytocin, Ml/min 100 g, Each electrode	Mean	After oxytocin, Ml/min 100 g, Each electrode	Mean	In percent of the flow before oxytocin, Each electrode	Mean
13	37	Resection of the Fallopian tubes	3	49.5 72.6	61.1	13.9 40.8	27.4	27.1 56.2	41.7						
14	22	Cervicitis	3	172.5 181.9	177.2	1.3 1.3	1.3	0.8 0.7	0.8	39.6	39.6	47.8	47.8	120.7	120.7
15*	28	Resection of the Fallopian tubes	5	130.8 173.3	152.1	3.8 8.0	5.9	2.9 4.6	3.8	99.0 86.7	92.9	56.3 54.6	55.5	56.9 63.0	60.0
16	21	Uterine myoma	7	112.3 148.4	130.4	21.3 58.0	39.7	19.0 39.1	29.1						
17**	25	Intermenstr bleeding	8	99.0 83.5	91.3	5.6 13.9	9.8	5.7 16.6	11.2	106.6 121.6	114.1	85.6 50.9	68.3	80.3 41.9	61.1
18	32	Intermenstr bleeding	9	77.0 90.0	83.5	3.8 4.5	4.2	4.9 5.0	5.0	34.7	34.7	33.0	33.0	95.1	95.1
19	23	Intermenstr bleeding	—	90.0 73.0	81.5	1.9 3.1	2.5	2.1 4.2	3.2	86.6 52.1	69.4	38.5 40.1	39.3	44.5 77.0	60.8
20	27	Carcinoma of the cervix, stage 0	—	31.5 33.5	32.5	7.2 5.9	6.6	22.7 17.6	20.2	86.6 115.5	101.1	22.4 45.3	33.9	25.9 39.2	32.6
21	40	Resection of the Fallopian tubes	9	112.3 130.0	121.2	34.7 57.7	46.2	30.9 44.4	37.7						
22	36	Resection of the Fallopian tubes	3	77.0	77.0	27.7	27.7	36.0	36.0						
Mean S.E.					100.8 ±13.88		17.1 ±3.26		18.9 ±5.00		73.3		46.3		71.7 ±12.75

curves from these patients were mono-exponential or had the same shape before and after injection of oxytocin and showed no flow changes during the desaturation time

DISCUSSION

Mean myometrial blood flow in women with normal ovulatory menstrual cycles and in postmenopausal women was of the same magnitude as observed previously (8), but the values were considerably higher than measured by Xe^{133}-clearance during gynaecological laparotomies (6). The significantly lower myometrial blood flow in postmenopausal women than in women with normal menstrual cycles might probably be rather due to retrogressive histological changes in the uterus

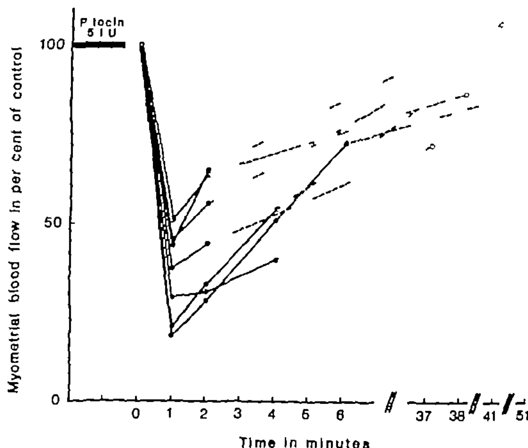


Fig 2 Effect of oxytocin on myometrial blood flow in per cent of control at different time. Seven electrodes in 4 patients. The patients are included in Table II (No. 21) and in Table III (Nos. 30, 32 and 33)

plots of the desaturation curves. The straight lines show the slopes used for flow calculation. After oxytocin was given (lower part) the semi-logarithmic plots of myometrial desaturation showed a slight curving with the convexity upwards, while the plots from cervical desaturation had about the same shape as before Myometrial flow was calculated from the tangents of the plots.

From 7 electrodes in 4 women myometrial blood flow was measured before and immediately after the injection of oxytocin and an additional measurement was performed 40–50 min after the administration of oxytocin with the electrodes in the same position. Fig. 2 demonstrates that even if blood flow decreased considerably during the first minutes after injection of oxytocin, it had risen to control values again 40–50 min later

Table II shows that myometrial blood flow two minutes after injection of oxytocin was reduced to an average of 18 9 $\pm$ 5 08 % of controls in ten women in the proliferative phase. The reduction of mean cervical blood flow was much less. At ten electrode sites in six women blood flow decreased to an average of 71 7 $\pm$ 12.75 % of controls. The difference between the effect of oxytocin on myometrial and cervical blood flow was found to be statistically significant ($p = 0.01$).

Myometrial blood flow in women in the secretory phase (Table III) had a similar reaction to oxytocin as in the proliferative phase. An average myometrial flow reduction to 33 1 $\pm$ 7 62 % of controls was found. In this group cervical flow was reduced to a mean of 73 5 $\pm$ 6.78 % of controls at nine electrode sites in five women The difference between changes in myometrial and cervical blood flow was found to be statistically significant ($p = 0.02$).

Both in the proliferative and the secretory phase blood flow in some cases remained reduced to 10–15 % of controls during the first ten minutes after injection of oxytocin.

When oxytocin was given intravenously to 10 postmenopausal women (Table IV) before the second blood flow measurement, mean myometrial blood flow was reduced to 94.3 $\pm$ 4 71 % of flow before the injection The hydrogen desaturation

Table II. *Effect of oxytocin on uterine blood flow proliferative phase*

Pt. no.	Age (y)	Diagnosis	Day after last menstr. period	Myometrial blood flow						Cervical blood flow					
				Before oxytocin		Two minutes after oxytocin				Before oxytocin		After oxytocin			
				Ml/min 100 g		Ml/min 100 g		In percent of the flow before oxytocin		Ml/min 100 g		Ml/min 100 g		In percent of the flow before oxytocin	
				Each electrode	Mean	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean
13	37	Resection of the Fallopian tubes	3	49.5 72.6	61.1	13.9 40.8	27.4	27.1 56.2	41.7						
14	22	Cervicitis	5	172.5 181.9	177.2	1.3 1.3	1.3	0.8 0.7	0.8	39.6	39.6	47.8	47.8	120.7	120.7
15*	28	Resection of the Fallopian tubes	5	130.8 173.3	152.1	3.8 8.0	5.9	2.9 4.6	3.8	99.0 86.7	92.9	56.3 54.6	55.5	56.9 63.0	60.0
16	28	Uterine myoma	7	112.3 148.4	130.4	21.3 58.0	39.7	19.0 39.1	29.1						
17**	23	Intermenstr bleeding	8	99.0 83.5	91.3	5.6 13.9	9.8	5.7 16.6	11.2	106.6 121.6	114.1	85.6 50.9	68.3	80.3 41.9	61.1
18	32	Intermenstr bleeding	9	77.0 90.0	83.5	3.8 4.5	4.2	4.9 5.0	5.0	34.7	34.7	33.0	33.0	95.1	95.1
19	28	Intermenstr bleeding	—	90.0 73.0	81.5	1.9 3.1	2.5	2.1 4.2	3.2	86.6 52.1	69.4	38.5 40.1	39.3	44.5 77.0	60.8
20	27	Carcinoma of the cervix, stage 0	—	31.5 33.5	32.5	7.2 5.9	6.6	22.7 17.6	20.2	86.6 115.5	101.1	22.4 45.3	33.9	25.9 39.2	32.6
21	40	Resection of the Fallopian tubes	9	112.3 130.0	121.2	34.7 57.7	46.2	30.9 44.4	37.7						
22	36	Resection of the Fallopian tubes	3	77.0	77.0	27.7	27.7	36.0	36.0						
Mean S.E.					100.8 ±13.89		17.1 5.26		18.9 ±5.08		75.3		46.3		71.7 ±12.75

curves from these patients were mono-exponential or had the same shape before and after injection of oxytocin and showed no flow changes during the desaturation time.

DISCUSSION

Mean myometrial blood flow in women with normal ovulatory menstrual cycles and in postmenopausal women was of the same magnitude as observed previously (8), but the values were considerably higher than measured by Xe^{133}-clearance during gynaecological laparotomies (6). The significantly lower myometrial blood flow in postmenopausal women than in women with normal menstrual cycles might probably be rather due to retrogressive histological changes in the uterus

Table III. *Effect of oxytocin on human uterine blood flow secretory phase*

Pat. no.	Age (y)	Diagnosis	Day after last menstr period	Myometrial blood flow: Before oxytocin, Ml/min 100 g, Each electrode	Myometrial: Before oxytocin, Ml/min 100 g, Mean	Myometrial: Two minutes after oxytocin, Ml/min 100 g, Each electrode	Myometrial: Two minutes after oxytocin, Ml/min 100 g, Mean	Myometrial: Two minutes after oxytocin, In percent of the flow before oxytocin, Each electrode	Myometrial: In percent of the flow before oxytocin, Mean	Cervical blood flow: Before oxytocin, Ml/min 100 g, Each electrode	Cervical: Before oxytocin, Ml/min 100 g, Mean	Cervical: After oxytocin, Ml/min 100 g, Each electrode	Cervical: After oxytocin, Ml/min 100 g, Mean	Cervical: After oxytocin, In percent of the flow before oxytocin, Each electrode	Cervical: In percent of the flow before oxytocin, Mean
23	28	Resection of the Fallopian tubes	17	55.4 130.8	93.1	18.7 34.7	26.7	33.8 26.5	30.2	51.3	51.3	44.7	44.7	87.1	87.1
4	45	Uterine myoma	18	173.3 138.6	156.0	10.7 10.7	10.7	6.2 7.7	7.0	37.0 51.0	44.0	[illegible] 37.5	31.4	68.1 73.5	70.8
5	39	Occlusion of the Fallopian tubes	20	188.8 173.3	181.1	116.0 40.8	78.4	61.4 23.5	42.5						
26	29	Occlusion of the Fallopian tubes	20	149.1 134.7	141.9	80.2 63.0	71.6	53.8 46.8	50.3						
27	30	Infertility	20	89.4 67.6	78.5	29.5 19.2	24.4	33.0 28.4	30.7						
28	38	Uterine myoma	21	102.7 79.2	91.0	20.4 16.9	18.7	19.9 21.3	20.6	89.4 69.3	79.4	63.0 60	17	70.5 87.0	78.8
29	28	Infertility	23	115.6 74.3	95.0	2.4 2.7	2.6	2.1 3.6	2.9						
30	27	Occlusion of the Fallopian tubes	24	70.5 67.3	68.9	67.0 58.8	62.9	95.0 87.4	91.2						
31	25	Intermenstr bleeding	25	126.0 130.8	128.4	12.2 8.5	10.4	9.7 6.5	8.1						
32	26	Occlusion of the Fallopian tubes	26	74.5	74.5	48.5	48.5	65.1	65.1						
33	37	Intermenstr bleeding	26	118.8 109.5	114.2	76.0 61.6	68.8	64.0 56.3	60.2						
34	44	Intermenstr bleeding	28	33.0	33.0	1.0	1.0	3.0	1.0	27.7 14.4	21.1	20.4 13.1	16.8	73.6 91.0	82.3
35	27	Carcinoma the cervix stage 0	—	106.5	106.5	19.3	19.3	18.1	18.1	81.5 55.4	68.5	37.5 28.3	32.9	46.0 51.1	48.6
Mean ±S.E.					104.8 ±11.03		34.2 ±7.77		33.1 ±7.62		52.9		37.5		73.3 6.78

Table IV *Effect of oxytocin on myometrial blood flow in postmenopausal women*

Pat. no	Age (y)	Years since menopause	Before oxytocin: Ml/min 100 g, Each electrode	Before oxytocin: Ml/min 100 g, Mean	After oxytocin: Ml/min 100 g, Each electrode	After oxytocin: Ml/min 100 g, Mean	After oxytocin: % percent of the flow before oxytocin, Each electrode	After oxytocin: % percent of the flow before oxytocin, Mean
36	63	21	53.3 72.9	63.1	47.8 72.9	60.4	89.7 100.0	94.9
37	60	10	94.5 115.5	105.0	77.0 86.4	81.8	81.5 75.0	78.3
38	71	20	72.9 77.0	75.0	57.8 60.3	59.1	79.3 78.3	78.8
39	69	25	51.0 47.8	49.4	51.0 49.5	50.3	100.0 103.6	101.8
40	74	26	46.2 37.5	41.9	42.0 36.5	39.3	90.9 97.3	94.1
41	61	12	115.5	115.5	106.6	106.6	92.3	92.3
42	62	12	63.0	63.0	56.6	56.6	89.8	89.8
43	59	11	77.0 89.4	83.2	77.0 81.5	79.3	100.0 91.2	95.6
44	53	6	64.8 69.3	67.1	57.8 57.8	57.8	89.2 83.4	86.3
45	56	8	37.5 46.2	41.9	50.4 59.0	54.7	134.4 127.7	131.1
Mean S.E.				70.5 ±7.89		64.6 ±6.13		94.3 ±4.71

than to hormonal differences at the time of measurement.

In two subsequent cervical blood flow measurements with intervals of 20–30 min, a flow decrease of about 15% from the first to the second measurement has previously been demonstrated (7). About the same reduction was obtained for myometrial blood flow in the present study.

In most of the women who had normal ovarian menstrual cycles, oxytocin caused considerable decrease of myometrial blood flow although the scatter of flow reduction was wide. Large doses of oxytocin induce an initial fall in blood pressure (3, 9) apparently caused by vasodilatation (10). In the present study blood pressure was not recorded during the desaturation period, but it seems unlikely that a decrease in blood pressure could be responsible for the selective reduction in the myometrium compared to cervix. A similar effect would then be expected in the postmenopausal women, but in this group no effect of oxytocin was obtained.

It is generally accepted that oxytocin usually increases uterine activity during the first few days after termination of pregnancy. Dahle (5) demonstrated that posterior pituitary gland extract increased uterine activity both in vitro and in vivo, also in non-pregnant women. The effect of pitressin was stronger than that of oxytocin. Coutinho & Lopes (4) and Bengtsson (2) showed that one IU of oxytocin influenced myometrial activity very little or not at all, whereas vasopressin was a much more potent stimulant. The doses of oxytocin used in the present study were 5 times higher and might therefore have influenced the myometrial response. The clinical observation that women in the proliferative and secretory phase, who had a marked blood flow reduction after administration of oxytocin, also felt pains in the lower abdomen indicates that uterine contractions might be responsible for the flow effect. This explanation was supported by the considerable difference between the effect of oxytocin on myometrial and cervical blood flow in these groups. Even if a dissociation of the effect of oxytocin on myometrial and cervical arterioles from the branches of the uterine artery might be possible, it seems more likely that the flow reduction depends on external compression of blood vessels. The external compression of uterine vessels in the myometrium is supposed to be high due to myometrial contraction, whereas little

Table III *Effect of oxytocin on human uterine blood flow secretory phase*

				Myometrial blood flow						Cervical blood flow					
				Before oxytocin		Two minutes after oxytocin				Before oxytocin		After oxytocin			
			Day after last menstr period	Ml/min 100 g		Ml/min 100 g		In percent of the flow before oxytocin		Ml/min 100 g		Ml/min 100 g		In percent of the flow before oxytocin	
Pat. no.	Age (y)	Diagnosis		Each electrode	Mean	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean
23	28	Resection of the Fallopian tubes	17	55.4 130.8	93.1	18.7 34.7	26.7	33.8 26.5	30.2	51.3	51.3	44.7	44.7	87.1	87.1
24	45	Uterine myoma	18	173.3 138.6	156.0	10.7 10.7	10.7	6.2 7.7	7.0	37.0 51.0	44.0	[illegible]5.2 37.5	31.4	68.1 73.5	70.8
25	39	Occlusion of the Fallopian tubes	20	188.8 173.3	181.1	116.0 40.8	78.4	61.4 [illegible]3.5	42.5						
26	29	Occlusion of the Fallopian tubes	20	149.1 134.7	141.9	80.2 63.0	71.6	53.8 46.8	50.3						
27	30	Infertility	20	89.4 67.6	78.5	29.5 19.2	24.4	33.0 28.4	30.7						
28	38	Uterine myoma	21	102.7 79.2	91.0	20.4 16.9	18.7	19.9 21.3	20.6	89.4 69.3	79.4	63.0 60	[illegible]7	70.5 87.0	78.8
29	28	Infertility	23	115.6 74.3	95.0	2.4 [illegible]7	2.6	2.1 3.6	2.9						
30	27	Occlusion of the Fallopian tubes	24	70.5 67.3	68.9	67.0 58.8	62.9	95.0 87.4	91.2						
31	[illegible]5	Intermenstr bleeding	25	126.0 130.8	128.4	12.[illegible] 8.5	10.4	9.7 6.5	8.1						
3	26	Occlusion of the Fallopian tubes	26	74.5	74.5	48.5	48.5	65.1	65.1						
33	37	Intermenstr bleeding	26	118.8 109.5	114.2	76.0 61.6	68.8	64.0 56.3	60.2						
34	44	Intermenstr bleeding	28	33.0	33.0	1.0	1.0	3.0	3.0	27.7 14.4	21.1	20.4 13.1	16.8	73.6 91.0	8[illegible].3
35	27	Carcinoma the cervix stage 0	—	106.5	106.5	19.3	19.3	18.1	18.1	81.5 55.4	68.5	37.5 28.3	[illegible]9	46.0 51.1	48.6
Mean ±S.E.					104.8 ±11.03		34.2 ±7.77		33.1 ±7.62		52.9		37.5		73.5 6.78

12. Schwalm, H. & Dubrauszky, V.: The structure of the musculature of the human uterus—muscles and connective tissue. Amer J Obstet Gynecol 94: 391–404, 1966.

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increase in tissue pressure is to be expected in the cervix with its small amount of smooth muscle fibres.

If administration of oxytocin causes uterine activity changes of electrode position might be responsible for the blood flow variation. The increase of hydrogen desaturation in proportion to elapsed time after injection of oxytocin indicates that the electrodes still are positioned in the myometrium Since the stiff electrodes in the myometrium indirectly were fixed to the cervix, contractions of the uterus might move the electrode tips deeper into the myometrium. However when needles were introduced through the cervical canal into the myometrium no change in needle position in relation to the external os could be observed during the first few minutes after an intravenous injection of oxytocin It seems therefore likely that blood flow measurements were performed in the same area in the myometrium before and after administration of oxytocin This is also supported by the fact that when a third measurement in some cases was performed about half an hour after the injection of oxytocin blood flow again had risen considerably compared with flow immediately after the drug was given

In most cases the influence of oxytocin on blood flow was about the same at the two electrodes. The difference obtained in some cases might be due to different myometrial activity at different time in the two areas.

Mean blood flow measured in the uterine artery by electromagnetic flowmeter after administration of oxytocin in similar doses was reduced to about 65% of controls (9) while local blood flow in the myometrium decreased to 20–30% of controls. The higher effect of oxytocin on local myometrial blood flow compared with that on flow in the uterine artery depends probably on the fact that the uterine artery supplies both the myometrium and the cervix and in the latter tissue blood flow was only sligthly reduced In both groups the patients still had their menstrual periods, but the higher mean age in the first group might also perhaps contribute to the difference.

That the effect of oxytocin was about the same in the proliferative and the secretory phase is in agreement with the results from measurements of blood flow in the uterine artery obtained with electromagnetic flowmeter (9) The observations indicate that hormonal changes during the menstrual cycle do not seem to influence the reduction of flow However the absence of effect of oxytocin on uterine blood flow in the postmenopausal women compared with the marked effect in women with intact ovarian function indicates that there might be a connection between ovarian hormones and the effect of oxytocin on uterine blood flow It is probable that lower uterine activity due to both hormonal differences and retrogressive changes in the myometrium itself are responsible for the lack of effect in postmenopausal women. This assumption is supported by the observation of Schwalm & Dubrauszky (12) that the uterus contains significantly less muscle tissue after menopause than before.

ACKNOWLEDGEMENT

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REFERENCES

1 Aukland, K., Bower B. F & Berliner R. W. Measurement of local blood flow with hydrogen gas. Circ Res 14 164–187 1964
2. Bengtsson, L. Ph.. Effect of progesterone upon the in vivo response of the human myometrium to oxytocin and vasopressin. Acta Obstet Gynecol Scand 49 Suppl. 6, 19–25 1970.
3 Bieniarz, J From Discussion in Oxytocin (ed. R. Caldeyro-Barcia and H. Heller) pp 80–83 Pergamon Press, Oxford, 1961
4. Coutinho, E. M. & Lopes, A. C. V Response of the non-pregnant uterus to vasopressin as an index of ovarian function Amer J Obstet Gynecol 10 479–489 1968.
5 Dahle T The effect of posterior pituitary gland extract on the activity of the human uterus Acta Obstet Gynecol Scand 30 S ppl. 4 1950
6 Jansson, I 133Xenon clearance in the myometrium of pregnant and non-pregnant women Acta Obstet Gynecol Scand 48 302 321 1969
7 Klingenberg, I & Aukland, K Measurement of human uterine cervical blood flow by local hydrogen gas clearance Acta Obstet Gynecol Scand 48 455–469 1969
8 Klingenberg, I Measurement of blood flow in human myometrium by local hydrogen clearance Acta Obstet Gynecol Scand 48 470–478 1969
9 — Measurement of uterine blood flow in non-pregnant women by electromagnetic flowmeter In press
10 Pickford, Mary Some extra-uterine actions of oxytocin. I Oxytocin (ed. R. Caldeyro-Barcia and H. Heller) pp. 68–79. Pergamon Press, Oxford, 1961
11 Prill, H. J Über die Durchblutung des Uterus Z Geburtsh Gynaekol 152 180–202, 1959

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LUMBAR EPIDURAL ANALGESIA IN LABOUR

II. *Effects on Glucose, Lactate, Sodium, Chloride, Total protein, Haematocrit and Haemoglobin in Maternal, Fetal and Neonatal Blood*

B. Thalme, N. Raabe and P. Belfrage

From the Department of Paediatrics (Head: John Lind) and the Department of Obstetrics and Gynaecology (Head: Ulf Borell), Karolinska Sjukhuset, Stockholm, Sweden

Abstract. Thirty-three full-term nulliparae and their babies were studied. Twenty-one received lumbar epidural analgesia with bupivacaine (Marcain-adrenalin®) and twelve conventional obstetrical analgesia with meperidine (Pethidin®), chlorpromazine (Hibernal®), nitrous oxide and pudendal nerve block with prilocaine (Citanest®). Glucose, lactate, potassium, sodium, chloride, total protein, haemoglobin and haematocrit were determined in fetal and maternal blood during labour and in neonatal blood after birth. This study revealed a high level of plasma sodium in scalp blood of both 'epidural' and 'control' series probably related to water loss during labour. Furthermore, administration of epidural analgesia to women in labour resulted in lower lactate concentrations in these women and their babies compared with those receiving conventional obstetric analgesia.

Lumbar epidural analgesia for women in labour has been found to result in a lower degree of metabolic acidosis in mother and child than conventional obstetric analgesia and to have no harmful effects on the newborn infant (11). In the present study a number of chemical components were assayed in maternal, fetal and neonatal blood after epidural analgesia to the mother. The components were glucose, lactate, sodium, chloride, total protein, haematocrit and haemoglobin.

MATERIAL AND METHODS

The patients were divided into three groups. Groups I and II comprised the 24 patients presented in a previous report (11), Group I receiving epidural analgesia and Group II receiving conventional obstetric analgesia. During analysis of the electrolyte data of these two groups a surprisingly high level of plasma sodium was found in fetal scalp blood, the highest values being observed in the epidural group. In order to investigate this finding further an additional group of nine healthy nulliparous women receiving epidural analgesia was included (Group III). Clinical data on these mothers and their fetuses and newborns are presented in Tables I and II.

The analgesia methods, postnatal care, sampling procedures, time periods and statistics have been described earlier (11). Microtitre methods were used for determination of glucose, lactate, potassium, sodium, chloride and total protein (8, 10). Haemoglobin was determined as cyanmethaemoglobin according to Van Kampen and Zijlstra (12) and haematocrit on an International micro capillary centrifuge, Model MB. In patients Groups I and II glucose, lactate, potassium, sodium, chloride and total protein were determined and in patients in Group III haemoglobin, haematocrit, sodium, chloride, total protein and pH were determined. In Group III patients an additional blood sample was drawn immediately prior to administration of epidural analgesia at a cervical dilatation of about 4 cm (C_{4}).

The maternal and fetal levels of the chemical components did not differ significantly between the two groups of epidural patients (Group I and Group III) during labour. Therefore the results of Group I and Group III were combined and used for calculation of means and standard deviations.

RESULTS

The mean values and standard deviations for the constituents determined were grouped according to the time of sampling. The results are presented in Figs. 1–4.

Maternal blood

The infusion of about 400 cc 5% glucose to the "epidural" mothers resulted in a higher glucose level in these mothers as compared with

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The maternal and fetal levels of the chemical components did not differ significantly between the two groups of epidural patients (Group I and Group III) during labour. Therefore the results of Group I and Group III were combined and used for calculation of means and standard deviations.

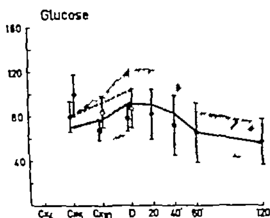
RESULTS

The mean values and standard deviations for the constituents determined were grouped according to the time of sampling. The results are presented in Figs. 1–4.

Maternal blood

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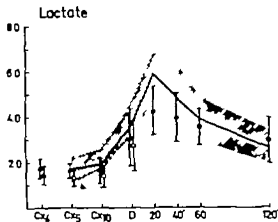


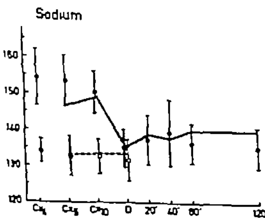
Fig. 1. Mean values ±1 S.D. of glucose and lactate in maternal (open circles), fetal and neonatal (closed circles) blood of the epidural group plotted against corresponding values of the control group. The dotted line represents maternal and the solid line fetal or neonatal mean values and the shaded area ±1 S.D. of the control group. The time axis illustrates the degree of cervical dilatation 4, 5 or 10 cm, D stands for delivery and 20, 40, 60 and 120 for min postnatally. Glucose (mg/100 ml) as determined in 12 "epidural" and 12 "control" patients and lactate (mEq/l) in 12 "epidural" and 10 "control" patients.

lactate levels tended to be lower than corresponding fetal levels. At Cx the maternal-fetal concentration differences were significant in the control group (0.06 mEq/l, $0.001 > p$).

The maternal concentration of *potassium*, *sodium*, *chloride* and *total protein* were similar in both "control" and "epidural" mothers and remained unchanged during labour except for a slight increase of total protein (0.52 g/100 ml, $0.05 > p > 0.01$) in "control" mothers. The maternal concentrations of *haemoglobin* and *haematocrit* in the epidural group were significantly below the levels in fetal scalp blood and remained fairly stable during the first stage of labour (Fig. 4). Between Cx and delivery a slight, probably significant, increase was observed for haemoglobin (0.65 g/100 ml, $0.05 > p > 0.01$) and for haematocrit (2.6 $0.05 > p > 0.01$).

Fetal blood

In the fetal scalp blood the concentrations of *glucose* and *lactate* remained fairly stable dur



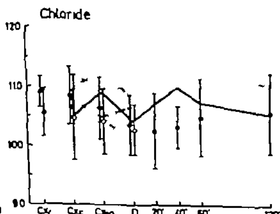


Fig. 2. Sodium and chloride in maternal and fetal blood during labour and in neonatal blood during the first 2 hours. The mean values ±1 S.D. in 15 patients of the epidural group are plotted against corresponding values in 12 patients of the control group. Sodium and chloride in mEq/l. Same legends as in *Fig. 1.*

Table I *Maternal data*

Case No.	Age	Oxytocin stimulation	Hypotension	Duration of: Labour[a] (h)	Duration of: Second stage (min)	Analgesia: Total dose buphvacaine (mg) (no. of doses)	Analgesia: Duration of epidural block (h): 1st dose-delivery	Analgesia: Duration of epidural block (h): Last dose-delivery	Vacuum extraction
E 25	25	+	−	8	60	38 (2)	1 7	1 1	−
E 26	25	−	−	6	35	33 (2)	3.1	0.6	+
E 27	30	+	−	26	40	35 (2)	5.1	1 7	−
E 28	29	+	+	7	60	65 (3)	5.0	1.2	+
E 29	26	+	+	10	60	40 (2)	3 0	1 0	+
E 30	38	−	−	8	75	23 (1)	2.8	2.8	+
E 31	30	+	−	23	60	63 (3)	8.8	1.8	+
E 32	22	+	−	9	75	35 (2)	4.0	1 7	+
E 33	26	+	−	11	65	18 (1)	1 6	1 6	−

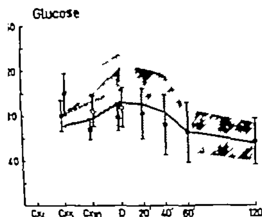
[a] Calculated from a cervical dilatation of 2 cm.

the "control" mothers at Cx_8 (difference 18.4 mg/100 ml $0.05>p>0.01$). The amount of infused glucose corresponds to 1 3 mg glucose per kg bw/min. No statistical relation was obtained between the acid-base parameters and the amount of infused glucose or individual glucose values. The maternal glucose level at Cx_{10} and at delivery did not differ significantly between the two groups, although it decreased in "epidurals" and increased in controls The mean decrease was 12.6 mg/100 ml ($0.05>p>0.01$) in epidural mothers and the mean increase 19 6 mg/100 ml ($0.05>p>0.01$) in "control" mothers. The maternal fetal mean differences of glucose at Cx_8 and Cx_{10} were highly significant ($0.001>p$) in both epidural and control groups and varied between 11 and 16 mg/100 ml. At delivery no significant maternal fetal mean difference was obtained in any group.

The concentration of *lactate* increased in both groups during labour but this increase was less pronounced in "epidural" mothers as compared with "control" mothers. For "control" mothers the mean difference between the values obtained at Cx_8 and delivery was 2.21 mEq/l ($0.001>p$) and corresponding figure for "epidural" mothers was 1.24 mEq/l ($0.01>p>0.001$). At Cx_8 the level of lactate was significantly correlated with corresponding P_{CO_2} level in "control" mothers ($n=10$ $r=0.80$ $0.01>p>0.001$). The maternal

Table II *Fetal and neonatal data*

Case No.	Gestational age (weeks)	Birth-weight (g)	Length (cm)	Fetal distress	Nuchal cord	Apgar score: < 8 at 1 min	Apgar score: > 8 at 5 min	Variations in: Blood chemistry	Variations in: Postnatal course
E 25	40	4 000	53	—	I	−	+	—	—
E 26	41	3 080	51	Bradycardia	—	−		Low pH at birth	—
E 27	40	2 960	50	Bradycardia	—	−		—	—
E 28	40	3 310	50	—	—	−		—	—
E 29	40	3 600	53	Foetal acidos	I	+		Low pH at birth	—
E 30	40	4 150	53	Bradycardia Tachycardia	—	−	+	—	—
E 31	41	3 800	53	—	—		+	—	—
E 32	40	3 010	50	—	—	−	+	—	—
E 33	40	3 500	51	—	—	−	+	Low pH at birth	—



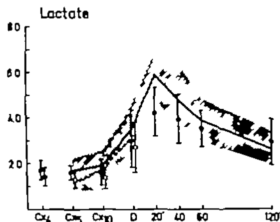


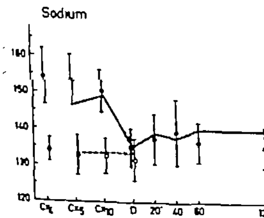
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lactate levels tended to be lower than corresponding fetal levels. At Cx_4 the maternal-fetal concentration differences were significant in the control group (0.06 mEq/l, $0.001 > p$).

The maternal concentration of *potassium sodium chloride* and *total protein* were similar in both "control" and "epidural" mothers and remained unchanged during labour except for a slight increase of total protein (0.52 g/100 ml, $0.05 > p > 0.01$) in "control" mothers. The maternal concentrations of *haemoglobin* and *haematocrit* in the epidural group were significantly below the levels in fetal scalp blood and remained fairly stable during the first stage of labour (Fig. 4). Between Cx_1 and delivery a slight, probably significant, increase was observed for haemoglobin (0.65 g/100 ml, $0.05 > p > 0.01$) and for haematocrit (2.6, $0.05 > p > 0.01$).

Fetal blood

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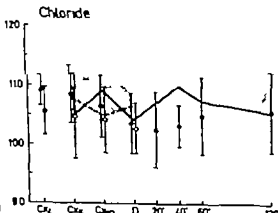


Fig. 2. Sodium and chloride in maternal and fetal blood during labour and in neonatal blood during the first 2 hours. The mean values ±1 S.D. in 15 patients of the epidural group are plotted against corresponding values in 12 patients of the control group. Sodium and chloride in mEq/l. Same legends as in Fig. 1.

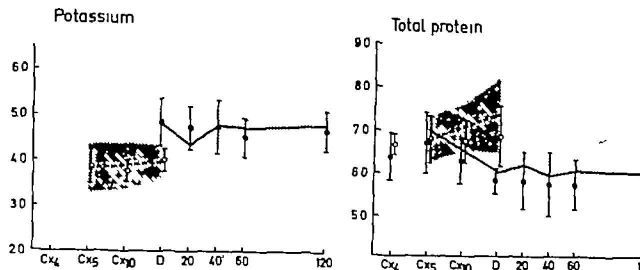


Fig 3 Potassium and total protein in maternal and fetal blood during labour and in neonatal blood *during* the first 2 hours. The mean values ±1 S.D in the epidural group are plotted against the corresponding values of the control group. Potassium was determined in 12 epidural and 1 control" mothers and total protein 20 *epidural and 12 control"* mothers. Potassium mEq/l, total protein in g/100 ml. Same legends as Fig. 1

ing labour in the epidural group but in the control group these components increased, the glucose with 20.2 mg/100 ml ($0.05 > p > 0.01$) and the lactate with 1.88 mEq/l ($0.001 > p$). The lactate values of the epidural series were probably significantly lower than in the control series at delivery (*difference 0.61 mEq/l, $0.05 > p > 0.01$*)

The *sodium* concentration in scalp blood was much higher than corresponding maternal blood. The maternal-fetal mean difference was similar and about 15 mEq/l in both groups, except f[illegible] an even larger difference at Cx_8 in the epidur[illegible] group. Comparison of the sodium level betwe[illegible] the epidural and the control groups showed [illegible] significant difference of 6.7 mEq/l ($0.01 > p$ [illegible] 0.001) at Cx_8. No differences in the sodium co[illegible]centrations were observed between the two grou[illegible] at Cx_{10} and delivery and no change occurred [illegible] the epidural group between Cx_4 and Cx_5

Due to the well known difficulties in obtai[illegible]

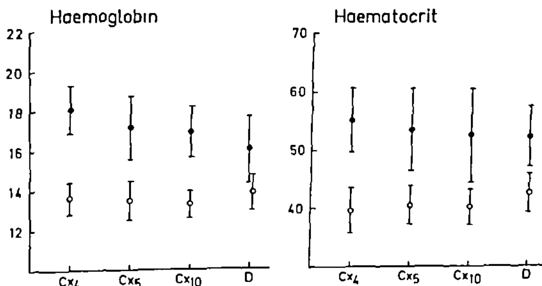


Fig 4 Haemoglobin and haematocrit in maternal and fetal blood during labour. The mean values ±1 *S D* in 9 patients of the epidural group. Haemoglobin in g 100 ml haematocrit in per cent packed red cell volume. Same legends as in *Fig 1*

ing a technical acceptable plasma sample for potassium analysis, no determinations of plasma potassium were carried out on fetal scalp blood. At delivery the concentration of plasma *potassium* in umbilical arterial blood was similar in "epidural" and "control" babies. The fetal levels of *total protein* decreased in both groups during labour but only in the epidural group, with its lower standard deviations, was this decrease found to be probably significant (difference Cx_6—delivery 0.82 g/100 ml 0.05 > p > 0.01). A slight decrease of *chloride* also occurred in the epidural group during labour (difference Cx_6—delivery 4.8 mEq/l, 0.05 > p > 0.01). The fetal levels of *haemoglobin* and *haematocrit* decreased gradually but not significantly throughout labour.

Neonatal blood

During the first two hours of extra-uterine life no differences in mean concentration of *glucose*, *lactate*, *potassium*, *sodium*, *chloride* and *total protein* were obtained between the epidural and the control group, except for lactate at 20 min (*difference* 1.61 mEq/l, 0.01 > p > 0.001) and for chloride at 40 min in "epidural" babies (difference 6.7 mEq/l, 0.05 > p > 0.01).

The concentrations of plasma sodium varied closely with the concentrations of haemoglobin and haematocrit in fetal scalp blood. The correlations between sodium and haematocrit ($n = 33$, $r = 0.40$; $0.05 > p > 0.01$) and between sodium and haemoglobin were significant ($n = 33$, $r = 0.50$; $0.01 > p > 0.001$). The latter correlation is also illustrated in Fig. 5. No such correlations were obtained between these components in maternal blood.

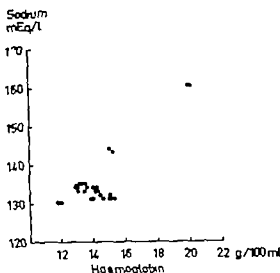


Fig. 5. Correlation between plasma sodium and haemoglobin in the epidural group. Closed circles represent fetal and open circles maternal values. (Correlation fetal scalp blood $n = 33$, $r = 0.50$; $0.01 > p > 0.001$.)

No significant changes occurred in pH, plasma electrolytes, haemoglobin or haematocrit in blood sampled prior to epidural analgesia (Cx_1) or 30 min later (Cx_2).

The close association of the metabolic component in the acid-base balance and the numerical difference between sodium and chloride in the Gamble diagram of plasma (2, 6) prompted us to calculate this difference. During labour the numerical difference between sodium and chloride concentrations in maternal plasma varied less in the epidural group than in the control group. Studying the individual changes of this numerical difference between Cx and delivery a mean increase of about 0.2 mEq/l was found in the "epidural" mothers compared to a mean decrease of about 1.9 mEq/l in the "control" mothers.

DISCUSSION

The levels of potassium, sodium, and chloride in maternal blood during labour and in neonatal blood during the first two hours after birth were similar in both epidural and control series and agreed well with previous reported results (5, 8).

The slight increase of total protein in the control mothers during labour probably reflected a minor loss of water by vaporization through lungs and skin. This might in turn be related to the pain-stimulated hyperventilation and sweating in women obtaining conventional obstetric analgesia (11).

The maternal glucose level was initially slightly higher in the epidural group, but during labour a gradual fall occurred, suggesting that the amount of infused glucose was too low to influence the blood glucose levels and the acid-base balance.

During labour blood can be obtained from the fetal scalp (7). This blood is not identical with the blood in the central circulation, one difference being the reduced amount of plasma water

in scalp blood (5) However the acid-base parameters in the scalp and the main systemic blood are well correlated (1) and this explains why determination of acid-base balance in scalp blood are reliable for assessing the condition of the fetus.

No intimate relation has been found for sodium or chloride between scalp and umbilical cord blood or between scalp and maternal venous blood (5) although it has been shown that these electrolytes are correlated in umbilical cord and maternal blood at midgestation and at term (8 9) The chloride concentration in fetal scalp blood in the present study remained fairly stable throughout labour in both groups and agreed well with the data of Jacobson (5) The levels of plasma sodium were surprisingly high in fetal scalp blood and differed significantly from the concentrations of plasma sodium in the umbilical artery and the maternal vein at delivery It seems difficult to find other explanations for the high concentration of plasma sodium in fetal scalp blood than a loss of water from the intravascular space. This was suggested by the high haemoglobin and haematocrit values and the intimate relation between these parameters and the sodium concentration. The loss of water might be caused by changes in the haemodynamics of the scalp due to molding of the foetal head during the process of birth.

The increase in maternal metabolic acidosis during labour has been attributed mainly to a concomitant increase in lactate as a result of the uterine activity and isometric contractions in other muscles (3 13). The rise of BD_{ma} was about 1 5 mEq/l in the "epidural" mothers and about 4 0 mEq/l in the "control" mothers (11) and the corresponding increase of lactate approximately 1.5 mEq/l and 2.5 mEq/l respectively This rise in lactate thus corresponded to the entire increase in metabolic acidosis in the epidural group and to about 60% in the control group. The remaining part of the metabolic acidosis in the control group could not be accounted for by lactate. One explanation might be the occurrence of a non-verified keto-acidosis, if the caloric intake in these patients did not meet the demand. No keto-acids were determined in the present study but some information might be gained from the changes in the sodium and the chloride concentrations during labour Production of keto-acids adds hydrogen to the plasma, causing a de crease in buffer base (= increase in BD_{ma}). A[n] estimation of buffer base can be obtained fro[m] the numerical difference between sodium an[d] chloride concentrations in plasma (6). A decrea[se] of the numerical difference between sodium an[d] chloride concentration would imply metaboli[c] acidosis and in the present study a decrease o[f] about 1 9 mEq/l occurred in maternal blood suggesting the presence of keto-acidosis in th[e] "control" mothers.

The levels of maternal lactate during labo[ur] agreed well with the results reported by Gemm[el] et al. (3), although in the present study highe[r] lactate concentrations were seen in women re ceiving conventional obstetric analgesia than i[n] those receiving epidural analgesia. The isometri[c] muscle contractions and hyperventilation with lo[w] P_{CO_2} levels in the "control" mothers undoubt edly resulted in increased production of materna[l] lactate (4). It is assumed that in this situatio[n] the normal elimination of lactate produced by the fetus is disturbed and in order to maintai[n] an adequate fetal maternal gradient the fetal lac tate level must rise. Consequently the observa tion of higher lactate concentration in fetal scalp blood of the "controls" than in the "epidurals" did not reflect intrauterine hypoxia but was as sociated with corresponding higher maternal lev els (8).

The lower lactate levels in both maternal and fetal blood during epidural analgesia also contributed to lower lactate levels in these babies at birth and also during the first 20 min of life In the "control" babies the postnatal lactate rise was higher and it took about one hour for this lactate level to drop to that of "epidural" babies. The lactate values of the present study and the acid-base data of the earlier study (11) suggest that epidural analgesia might be of benefit to the newborn infant. This assumption is based on the fetal maternal lactate gradient at low maternal lactate concentrations and the low lactate level at birth and immediately thereafter Due to these low lactate levels the time necessary for normalisation of the neonatal acid-base balance is shortened and thereby one parameter of the complex extrauterine adaptation is facilitated

This study shows a lower lactate concentration in women obtaining epidural analgesia and their babies compared with these obtaining conven-

tional obstetric analgesia. Furthermore a remarkably high level of plasma sodium in scalp blood is related to a water loss during labour and not to the type of analgesia.

ACKNOWLEDGEMENTS

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REFERENCES

1. Adamsons, K., Beard, R. W & Myers, R. E Comparison of the composition of arterial, venous and capillary blood of the foetal monkey during labour. Amer J Obstet Gynecol *107* 435, 1970
2. Gamble J L., Chemical Anatomy Physiology and Pathology of Extracellular Fluid. Harvard University Press, Cambridge, 1964.
3. Gemzell, C. A Robbe, H Stern, B. & Strom, G.: Observations on circulatory changes and muscular work in normal labour Acta Obstet Gynecol Scand *36* 75 1957
4. Huckabee, W E Relationships of pyruvate and lactate during anaerobic metabolism 1. Effects of infusion of pyruvate or glucose and of hyperventilation J Clin Invest *37* 244, 1958.
5. Jacobson, L Studies on Acid-base and Electrolyte Components of Human Foetal and Maternal Blood during Labour Studentlitteratur Lund, 1970
6. Rooth, G., Clinical Acid-base and Electrolyte Balance Studentlitteratur Lund, 1970.
7. Saling, E., Das Kind im Bereich der Geburtshilfe. Georg Thieme Verlag, Stuttgart, 1966.
8. Thalme, B Electrolyte and acid-base balance in fetal and maternal blood. An experimental and clinical study Acta Obstet Gynecol Scand *45* Supp. 8, 1967
9. — Potassium, sodium, chloride, total protein, calcium and inorganic phosphorus in plasma of human foetuses and newborn infants. Biol Neonat *18*. 444 1971.
10. Thalme, B., Engstrom, L., Broberger U Edstrom, K. & Kretschmar G., Infants of diabetic mothers. II. Acid-base and electrolyte balance during the first 48 hours after birth. Acta Paediat Scand *61* 315 1972.
11. Thalme, B., Belfrage, P & Raabe, N Lumbar epidural analgesia in labour I. Acid-base balance and clinical condition of mother fetus and newborn child. Acta Obstet Gynecol Scand *53* 27 1974.
12. Van Kampen, E. J & Zijlstra, W G Standardization of *hemoglobinometri. II. The hemoglobincyanid* method. Clin Chem Acta 6. 538, 1961
13. Wolf, H A comparative study of actual blood gases and acid base metabolism in maternal and foetal blood during parturition. *In* Intra-uterine Dangers to the Foetus (ed. J Horsky & Z. K. Stembera), p. 89 Excerpta Medica Foundation, Amsterdam, 1967

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DETERMINATION OF BIPARIETAL DIAMETER BY THE ULTRASONIC B-SCAN TECHNIQUE

K. Aantaa and M. Forss

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Abstract One thousand four hundred and forty-eight biparietal measurements were performed on 1 062 unselected patients between the 4th–42nd eeks of gestation 869 patients had single measurement and 193 patients had tw or more measurements. The B-scan method as used for the measurements The fetal age estimated from the biparietal measurement as compared ith the calculated maturity based on the last menstrual cycle

Questions to hich an answer has been sought through ultrasound studies during pregnancy include. Is the fetus too small or too large for the calculated gestation period? Does fetal de elopment ppear to have ceased? The A-scan technique has usually been employed to determine the biparietal diameter although the B-scan technique has been used initially to determine the position of the fetal head. Would it not be possible to determine the biparietal diameter equally well with the B-scan method? Finding an answer to this question was the motive for undertaking this study

MATERIAL

The series consisted of 1 062 patients on hom 1 448 measurements ere performed. In 869 of them the biparietal diameter as determined only once and in 193 tw or more times. The patients ere completely unselected and included cases ith toxemia, diabetes, blood incompatibility and breech presentation. All the pregnancies ere single

METHODS

The biparietal measurements ere performed ith Picker echo system model NDU-441 BU-B. A MH piezoelectric transducer was used. A longitudinal scan (Fig 1), scale 1 3 as taken first, hich revealed the position of the fetus and the fetal head. The transverse scan (Fig. 2), also scale 1 3 was made to obtain the midline echo. The scale was then changed to 1 2 to reduce the measuring error As far as possible all unnecessary echoes ere eliminated in this 1 2 picture (Fig. 3), leaving only the echoes from the skull bones and from the midline. In addition, an endeavour was made to obtain images that are thin enough to obviate difficulties in deciding hether the measurement as made from the inside or outside The number of pregnancies under 4 weeks was so small that they were disregarded. All the measurements ere made by one person

RESULTS

Using the measurements obtained, a mean and standard deviation s were calculated for each week from the formula

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n}}$$

in which $\bar{x}$ = mean, $x - \bar{x}$ = deviation of the ith measurement from the mean, n = number of measurements.

If the umber of measurements was smaller than 30 the divisor n in the formula was replaced by $n-1$ Table I gives the number of measurements made, the calculated means and the values of and $2s$. The results are also presented in Fig. 4. The shaded area represents the measurements that fall between the limits $\pm s$. The measurements within the $2s$ range are between the broken lines. 1 130 (78%) of the 1 448 measurements are within the range $\pm s$ and 1 369 (95%) within the range $\pm 2s$.



Fig 1 A longitudinal scan, scale 1 3

DISCUSSION

The growth of the biparietal measurement during the different phases of pregnancy has been studied by many authors. Biparietal measurements have generally been made with the A-scan technique Hellman and his co-workers (3) are of the opinion that the A-scan technique gives more accurate values than the B technique. An average weekly growth of 1 6 mm in the biparietal measurement during the last 10 weeks was reported by Willocks et al. (6). Thompson et al. (5) observed an average weekly growth of 1 8 mm in the biparietal diameter during the last trimester They considered that the growth curve tend to flatten towards the end. According to Camp bell (1), biparietal measurements made in th 20th–34th weeks of pregnancy are important a he stated, biologic variations in the growth c the fetal head are small up to the 30th wee of pregnancy and the fetal head grows rapidly Willocks and Dunsmore (7) reported that th growth curve for the biparietal diameter flatten towards the end of pregnancy The same resul was arrived at by Dewhurst et al. (?) accordin to whom the important point to emerge from this is that fetal head growth is almost linea



Fig 2 A t nsverse scan, scale 1 3



Fig. 3. A transverse scan, scale 1 2.

until 30 weeks, after which it is less rapid but more variable. Kratochwil (4) also concluded that information obtained towards the end of pregnancy from the biparietal measurement is more unreliable than during the 15th–20th weeks of pregnancy when the accuracy is of the order of 90% or during the 20th–30th weeks when it is of the order of 70%. In our study too the growth curve flattened especially during the last month of pregnancy. The weekly growth increment towards the end of pregnancy was 1.0 mm in our series. This is a smaller increment than has been reported by other authors (5 6). This may be accounted for by differing clinical material due to local variations. We used the B-scan technique for the biparietal measurement whereas the other researchers employed the A-scan method. Hellman and his co-workers (3) are of the opinion that the A-scan technique gives more accurate values than the B-scan technique. The former possibly gives more accurate values, but the B technique is quicker to use and the growth

Table I. *Increase in the biparietal diameter during the 24th–42nd weeks of pregnancy*

Week of pregnancy	Measurements	Biparietal diameter ($\bar{x}$) (cm)	(cm)	2s (cm)
24	16	5.6	±0.5	±1.0
25	14	6.2	±0.3	±0.6
26	1	6.6	±0.5	±1.0
27	17	6.9	±0.4	±0.8
28	26	7.2	±0.4	±0.8
29	22	7.6	±0.5	±1.0
30	41	7.9	±0.5	±0.8
[illegible]	23	8.1	±0.3	±0.6
[illegible]	68	8.3	±0.3	±0.6
[illegible]	75	8.5	±0.4	±0.8
34	97	8.7	±0.3	±0.6
35	94	8.9	0.3	±0.4
36	120	9.1	±0.3	±0.4
37	149	9.2	±0.3	±0.6
38	146	9.3	±0.3	±0.6
39	112	9.4	±0.3	±0.6
40	121	9.5	±0.2	±0.4
[illegible]	109	9.6	±0.2	±0.4
42	178	9.7	±0.2	±0.4

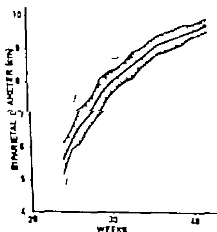


Fig. 4. Mean curve for the biparietal measurement. The shaded area represents the measurements that fall between the limits ±s; the measurements within the ±2s range are between the broken lines.



Fig 1 A longitudinal scan, scale 1 3

DISCUSSION

The growth of the biparietal measurement during the different phases of pregnancy has been studied by many authors. Biparietal measurements have generally been made with the A-scan technique Hellman and his co-workers (3) are of the opinion that the A-scan technique gives more accurate values than the B technique. An average weekly growth of 1 6 mm in the biparietal measurement during the last 10 weeks was reported by Willocks et al. (6). Thompson et al. (5) observed an average weekly growth of 1 8 mm in the biparietal diameter during the last trimester They considered that the growth curve tends to flatten towards the end. According to Campbell (1) biparietal measurements made in the 20th–34th weeks of pregnancy are important as, he stated, biologic variations in the growth of the fetal head are small up to the 30th week of pregnancy and the fetal head grows rapidly. Willocks and Dunsmore (7) reported that the growth curve for the biparietal diameter flattens towards the end of pregnancy The same result was arrived at by Dewhurst et al. (2) according to whom the important point to emerge from this is that fetal head growth is almost linear



Fig A transverse scan, scale 1 3

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LAPAROSCOPY VERSUS CULDOSCOPY IN THE INVESTIGATION OF INFERTILITY

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Abstract Some advantages and disadvantages of laparoscopy and culdoscopy are illustrated in a study on 174 infertile cases divided into three groups. The three groups were similar in relation to age, number of previous pregnancies and duration of infertility The first group comprises 52 women who underwent culdoscopy the second 49 cases who underwent laparoscopy Unexpectedly more cases of endometriosis were found in the culdoscopy group whereas adhesions were more frequent in the laparoscopy group. This was interpreted to be due to unawareness of the fact that endometriosis is more often situated in the lower pelvis whereas adhesions may be found also in the upper pelvis The third group of cases comprises 73 laparoscopies undertaken after reaching the above conclusions Consequently the ovaries were examined with more care than before and endometriosis was diagnosed more frequently These findings should remind the laparoscopist that endometriosis is more often present in the underside of the ovary

In the investigation of infertility the value of endoscopy in the form of culdoscopy or laparoscopy has become appreciated more and more by the clinician Although the frequency of unexpected pelvic pathology revealed by these methods cannot be accurately stated because the principles of patient selection vary from place to place, there is no doubt that the incidence is high enough to warrant endoscopy as part of the investigatory routine (1 3 5 7 8) The use of the laparoscope seems to be increasing but whether laparoscopy or culdoscopy is preferred may be more a matter of tradition and personal inclination than a choice based on individual clinical circumstances As the advocates of each method apparently detect pelvic pathology in about the same proportion of patients a comparison between the methods may seem not to be worth while However Roland (5) states that the two methods are not competitive but complementary as they do not reveal exactly the same parts of the peritoneal cavity and a comparison of the results obtained with the two methods is of some interest as it reveals pitfalls in the evaluation of the findings.

MATERIAL AND METHODS

The patients at the Fertility Clinic of the Karolinska Hospital were selected in various ways. During the years 1963 to 1971 fertility investigations were initiated or continued in a total of 498 infertile cases. A considerable number of them were referred to the clinic because the initial investigation did not reveal the cause of infertility Thus, when the patients were first seen by the author there was a great diversity in the previous fertility investigations In some cases a very extensive investigation had been carried out, including culdoscopy or laparoscopy; in other cases only a sperm count and hysterosalpingography had been performed and in some cases this was their first visit to the doctor

In those cases where the previous investigation had not revealed the cause of infertility and culdoscopy or laparoscopy had not been performed, one or other of these latter procedures was employed if the infertility had persisted for at least 3 years, or even earlier if the history or other clinical findings gave some suspicion of pelvic pathology The peritoneoscopies were performed by several doctors on the staff including the author

Culdoscopy was carried out under local anaesthesia using a Storz instrument The Fallopian tubes were not insufflated. For fuller inspection of the pelvis the genital organs and the intestines were redistributed by manipulation of the tenaculum on the portio and by external pressure on the abdomen The peritoneum of the cul de sac, the posterior aspect of the broad ligaments, and the ovaries were easily exposed in this way The tubes were frequently not visible along their whole course but the infundibulum could generally be inspected

Laparoscopy was performed under general anaesthesia using a Storz instrument. Frequently pertubation with

curve obtained with it has proved serviceable for our purposes.

CONCLUSIONS

The use of the B-scan ultrasound technique has proved to be practicable for determination of the biparietal diameter It is simple and quick. It involves no danger to the patient and is easy to repeat. The greater the number of determinations that can be made for the same patient, the more reliable is the result.

REFERENCES

1 Campbell S. J Obstet Gynaecol Br Comm 76 [illegible] 1969
2. Dewhurst, C. J Beazley J M & Campbell, S Am J Obstet Gynecol *113* 141 1972.
3 Hellman, L. M Kobayashi, M Fillisti, L. & Lavenhar M Amer J Obstet Gynecol 99 [illegible], 1967
4. Kratochwil, A Perinatale Medizin Bd *II* 47 [illegible]
5 Thompson, H. E., Holmes, J H Gottesfeld, K. R. & Taylor E. S. Amer J Obstet Gynecol 9[illegible] 44, 196[illegible]
6 Willocks, J Donald, I., Duggan T C. & Day N J Obstet Gynaecol Br Comm *71* 11, 1964.
7 Willocks, J & Dunsmore I R J Obstet Gynaecol Br Comm *78* 804 1971

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Table III. *Age and duration of infertility at the different clinical findings*

Findings	Mean age at investigation	Mean duration of infertility (years)
Normal findings (prim. infertility)	29.1	4.0
Normal findings (sec. infertility)	30.7	3.8
Endometriosis	30.3	4.6
Other pathology	29.0	4.8

in incidence in the two groups of patients. By culdoscopy the posterior aspects of the broad ligaments and the caudal circumference of the ovaries are viewed direct and without difficulty. The upper surface of the ovary is, however, sometimes difficult to bring into the field of view and it may also be difficult to visualize the Fallopian tubes completely. By laparoscopy on the other hand, the cranial circumference of the ovary is viewed together with the Fallopian tube without particular difficulty but the caudal pole of the ovary is out of sight. By the aid of manipulators it is possible to change the position of the adnexa, making the evaluation of the infundibulum of the tube more easy. In these circumstances, if the tubes appear normal and the ovaries are of normal size the investigator may be satisfied and believe the pelvis to be normal although the investigation has been incomplete. If all aspects of the ovary cannot easily be viewed it may be because of adhesions caused by endometriosis.

This situation in an infertility case may warrant laparotomy. The necessity to visualize the ovaries completely has recently been stressed by Samuelsson & Sjövall (6).

Another example of the difference between culdoscopy and laparoscopy in this study is that adhesions to the Fallopian tubes were less frequently observed by culdoscopy than by laparoscopy. Thus, pathological conditions in the upper pelvis are easily overlooked during culdoscopy whereas the lower pelvis may be inadequately examined by laparoscopy.

During the third period of investigation the results of the previous evaluations were known to the staff of the clinic. This knowledge may have increased the interest for the laparoscopic procedure as such, and the number of laparoscopies on the indication of infertility is consequently higher. We may also assume that the interest toward ovarian endometriosis and its laparoscopic diagnosis had increased. We would therefore expect that the findings in the third group better represent the true situation than before.

If the drawbacks of the two methods are of equal significance, the true frequency of endometriosis in relation to other pelvic pathology would be close to the mean of the frequencies observed in the first two groups. The relative frequency of endometriosis in the third group does approximate to this mean (Table II).

The macroscopic diagnosis of endometriosis is frequently regarded with some degree of scepticism. In the present study 26 cases were diagnosed in this way. In 16 cases the diagnosis of endometriosis was verified at operation and subsequent pathologic examination. In 1 case a sactosalpinx was wrongly interpreted as endometriosis at culdoscopy and in another case a laparoscopic diagnosis of sactosalpinx was at operation found to be an ovarian endometriotic cyst. In the other 8 cases the changes were not considered extensive enough to make laparotomy necessary.

The discrepancy in the frequency of the finding of endometriosis during the first two periods indicates that endometriosis is not by chance distributed over the surface of the ovary but that it is more frequently localized to the caudal surface, facing the cul de sac. There are many hypotheses concerning the development of endometriosis (4). It may be concluded from the findings in the present study that the theory of implantation is of major clinical significance. If viable endometrium is transported in a retrograde direction through the Fallopian tubes into the abdominal cavity it would sink downwards and could then under special conditions implant in the true pelvis. If the endometrium implants on the ovaries, implantation would preferably take place on the caudal surface.

REFERENCES

1. Dillon, T. F. & Smith, B. D.: Culdoscopy. Fertil Steril 21: 193–200, 1970.
2. Nordvall, S.: An instrument to aid laparoscopic diagnosis. Acta Obstet Gynecol Scand 49: 111–112, 1970.
3. Peterson, E. P. & Behrman, S. J.: Laparoscopy of the infertile patient. Obstet Gynecol 36: 363, 1970.

Table I *Composition of clinical material*

Group	Mean age at investigation	Mean duration of infertility (years)	Frequence of sec. infertility (%)
A. Culdoscopy group	29 6	4.2	14 9
B. Laparoscopy group I	28.7	3 7	16 6
C. Laparoscopy group II	29 7	4 6	18.3
Total	29.3	4.2	16 9

methylthionine solution, 0 1 mg/ml, was employed to assure tubal patency One or two manipulators (2) were inserted separately through the abdominal wall to facilitate the inspection.

In those cases where pathological conditions of probable significance for the infertility were revealed, laparotomy was performed and the accuracy of the endoscopic findings could be assessed. In cases of doubtful pathology surgical intervention was usually postponed for about one year

The study covers three periods, the first two of approximately equal length 3 years each. During the first period, mainly culdoscopy was employed, and during the second and third, laparoscopy The change in technique was mainly due to the acquisition of a cold-light laparoscope At the end of the second period our experience with the two techniques was evaluated During the third period the experience from this evaluation was put into practice The age distribution duration of infertility and frequency of secondary infertility were the same during the three periods (Table I).

RESULTS AND DISCUSSION

By mid 1969 about the same number of culdoscopies and laparoscopies had been performed in the investigation of hitherto unexplained infertility It was then considered appropriate to perform a retrospective analysis, comparing the two techniques. As the advantages of peritoneoscopy in infertility investigations were already well recognised and the comparison was retrospective it seems reasonable to assume that attitudes towards the investigation did not change, invalidating a comparison between the two periods. In favour of this assumption is the fact that the same number of investigations were performed during periods one and two which were of equal length. Also the two periods may be considered equivalent with respect to technical knowledge and experience as is reflected by the same order of technical failures (Table II). The findings were not influenced by age or duration of infertility (Table III).

When comparing the results of culdoscopy and laparoscopy during the first and second periods, we should expect the same incidence of pathological conditions and also the same spectrum of diagnoses. Culdoscopy revealed pelvic pathology in 34 6% of the cases, whereas abnormalities were found at laparoscopy in only 24.5% This difference, although not significant statistically may reflect a tendency to earlier investigation by peritoneoscopy in the second series (Table I). There is a more striking difference in the diagnosis of endometriosis during the two periods. By culdoscopy 13 cases of endometriosis were revealed, by laparoscopy 3 only Statistical analysis shows this difference to be significant ($\chi^2 = 5.63$ $p<0.02$)

These differences are probably due more to the procedure employed rather than real differences

Table II. *Findings at peritoneoscopy*

Figures within parentheses are the percentage values

Technique	Normal condition	Endometriosis	Other pelvic pathology	Quotient endometriosis other pelvic pathology	Technical failures	Total
A Culdoscopy	31 (59.6)	13 (25 0)	5 (9 6)	2.60	3 (5 8)	52
B. Laparoscopy (period 1)	35 (71 4)	3 (6.1)	9 (18 4)	0.33	2 (4 1)	49
C. Laparoscopy (period 2)	52 (71.2)	10 (13.7)	8 (11.0)	1.25	3 (4.1)	73
A + B	66 (65.3)	16 (15 8)	14 (13.9)	1 12	5 (5 0)	101
Total A + B + C	118 (67 9)	26 (14 9)	22 (12.6)	1 18	8 (4 6)	174

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During the third period of investigation the results of the previous evaluations were known to the staff of the clinic. This knowledge may have increased the interest for the laparoscopic procedure as such, and the number of laparoscopies on the indication of infertility is consequently higher. We may also assume that the interest toward ovarian endometriosis and its laparoscopic diagnosis had increased. We would therefore expect that the findings in the third group better represent the true situation than before.

If the drawbacks of the two methods are of equal significance, the true frequency of endometriosis in relation to other pelvic pathology would be close to the mean of the frequencies observed in the first two groups. The relative frequency of endometriosis in the third group does approximate to this mean (Table II).

The macroscopic diagnosis of endometriosis is frequently regarded with some degree of scepticism. In the present study, 26 cases were diagnosed in this way. In 16 cases the diagnosis of endometriosis was verified at operation and subsequent pathologic examination. In 1 case a sactosalpinx was wrongly interpreted as endometriosis at culdoscopy and in another case a laparoscopic diagnosis of sactosalpinx was at operation found to be an ovarian endometriotic cyst. In the other 8 cases the changes were not considered extensive enough to make laparotomy necessary.

The discrepancy in the frequency of the finding of endometriosis during the first two periods indicates that endometriosis is not by chance distributed over the surface of the ovary, but that it is more frequently localized to the caudal surface, facing the cul de sac. There are many hypotheses concerning the development of endometriosis (4). It may be concluded from the findings in the present study that the theory of implantation is of major clinical significance. If viable endometrium is transported in a retrograde direction through the Fallopian tubes into the abdominal cavity it would sink downwards and could then under special conditions implant in the true pelvis. If the endometrium implants on the ovaries, implantation would preferably take place on the caudal surface.

REFERENCES

1. Dillon, T. F. & Smith, B. D. Culdoscopy. Fertil Steril 21: 193–200, 1970.
2. Nordvall, S. An instrument to aid laparoscopic diagnosis. Acta Obstet Gynecol Scand 49: 111–112, 1970.
3. Peterson, E. P. & Behrman, S. J. Laparoscopy of the infertile patient. Obstet Gynecol 36: 363, 1970.

Table I *Composition of clinical material*

Group	Mean age at investigation	Mean duration of infertility (years)	Frequence of sec. infertility (%)
A. Culdoscopy group	29.6	4.2	14.9
B. Laparoscopy group I	28.7	3.7	16.6
C. Laparoscopy group II	29.7	4.6	18.3
Total	29.3	4.2	16.9

methylthionine solution, 0.1 mg/ml was employed to assure tubal patency. One or two manipulators (2) were inserted separately through the abdominal wall to facilitate the inspection.

In those cases where pathological conditions of probable significance for the infertility were revealed, laparotomy was performed and the accuracy of the endoscopic findings could be assessed. In cases of doubtful pathology surgical intervention was usually postponed for about one year.

The study covers three periods, the first two of approximately equal length, 3 years each. During the first period, mainly culdoscopy was employed, and during the second and third, laparoscopy. The change in technique was mainly due to the acquisition of a cold-light laparoscope. At the end of the second period our experience with the two techniques was evaluated. During the third period the experience from this evaluation was put into practice. The age distribution, duration of infertility and frequency of secondary infertility were the same during the three periods (Table I).

RESULTS AND DISCUSSION

By mid-1969 about the same number of culdoscopies and laparoscopies had been performed in the investigation of hitherto unexplained infertility. It was then considered appropriate to perform a retrospective analysis, comparing the two techniques. As the advantages of peritoneoscopy in infertility investigations were already well recognised and the comparison was retrospective it seems reasonable to assume that attitudes towards the investigation did not change, invalidating a comparison between the two periods. In favour of this assumption is the fact that the same number of investigations were performed during periods one and two, which were of equal length. Also the two periods may be considered equivalent with respect to technical knowledge and experience as is reflected by the same order of technical failures (Table II). The findings were not influenced by age or duration of infertility (Table III).

When comparing the results of culdoscopy and laparoscopy during the first and second periods, we should expect the same incidence of pathological conditions and also the same spectrum of diagnoses. Culdoscopy revealed pelvic pathology in 34.6% of the cases, whereas abnormalities were found at laparoscopy in only 24.5%. This difference, although not significant statistically, may reflect a tendency to earlier investigation by peritoneoscopy in the second series (Table I). There is a more striking difference in the diagnosis of endometriosis during the two periods. By culdoscopy 13 cases of endometriosis were revealed, by laparoscopy 3 only. Statistical analysis shows this difference to be significant ($\chi^2 = 5.63$, $p < 0.02$).

These differences are probably due more to the procedure employed rather than real differences

Table II. *Findings at peritoneoscopy*

Figures within parentheses are the percentage values

Technique	Normal condition	Endometriosis	Other pelvic pathology	Quotient endometriosis other pelvic pathology	Technical failures	Total
A. Culdoscopy	31 (59.6)	13 (25.0)	5 (9.6)	2.60	3 (5.8)	52
B. Laparoscopy (period 1)	35 (71.4)	3 (6.1)	9 (18.4)	0.33	2 (4.1)	49
C. Laparoscopy (period 2)	52 (71.2)	10 (13.7)	8 (11.0)	1.25	3 (4.1)	73
A + B	66 (65.3)	16 (15.8)	14 (13.9)	1.12	5 (5.0)	101
Total A + B + C	118 (67.9)	26 (14.9)	[illegible] (12.6)	1.18	8 (4.6)	174

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THE COURSE AND OUTCOME OF PREGNANCY IN WOMEN WITH NEUROSES

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Abstract. Data derived from the medical registration of births in Norway during 1967 and 1968 have been utilized in a study of the course and outcome of 388 pregnancies in women reporting to suffer from conditions classifiable as neuroses. Comparison is made with 112 530 pregnancies in women reporting no disease.

It was found that women with neuroses more often experience pregnancy complications, labour difficulties and premature labour and that their babies have a higher mortality and a higher frequency of congenital malformation, low birth weight and hypoxia.

The findings suggest that comprehensive care, both psychiatric and obstetric, is needed to lower the risk in these women and their infants.

Many studies have suggested an association between the emotional state of the mother and obstetric complications. An association has been also claimed with respect to the condition of their infants. Most of the studies are based on comparisons between the psychological characteristics of women experiencing obstetric complications and those of women whose pregnancy and labour were uncomplicated. Studies have dealt with obstetric complications in general (9, 16, 17, 18), or with specified complications such as hyperemesis (8, 13, 1), toxaemia (19, 20); habitual abortion (11, 15); premature labour (6); prolonged labour (2?), low birth weight (1). No specific personality pattern has been demonstrated in women who experience obstetric complications. However, of the various psychological characteristics, a high level of anxiety has quite consistently been noted.

The present study differs in two main respects from most of those referred to. Firstly it explores the obstetric complications which are more likely to occur in women having an emotional condition classifiable as neurosis. Secondly it comprises material collected over 2 years on a nation-wide scale (5). The material, made available through the Medical Birth Registry of Norway, has been used also in the study of the effect of other maternal diseases on the course and outcome of pregnancy (2, 3, 4).

MATERIAL AND METHODS

Medical registration of births is compulsory in Norway: midwives and physicians attending the delivery of a fetus of 16 weeks or more of gestation are required to complete a standard registration form. This form includes, in addition to the usual civil data, information on the mother's health before and during the pregnancy, as well as on labour and condition of the newborn. Copies of the registration forms are sent to local health authorities to serve as a means of communication between the obstetric and child health services. The original form is forwarded to the Medical Birth Registry for central processing and analysis (5).

The material from the years 1967 and 1968 includes a total of 134 368 pregnancies, of which 388 (2.9 per 1 000) were in women reporting to suffer from conditions classifiable as neuroses, and starting either before or during the pregnancy. According to the International Classification of Diseases (26), neuroses include conditions such as anxiety, hysteria, phobia, psychogenic depression, nervous debility, hypochondriasis, occupational neurosis, and nervous breakdown. The neurosis group is compared with a control group of 11 530 pregnancies in women reporting no disease before or during the pregnancy.

The group of women with neuroses and the control group were found to be comparable as regards parity: primiparae constituted 35.7% of the former group and 37.0% of the latter. Age distributions indicated that women with neuroses had a mean age of 29.6 years compared with 27.0 years for the controls.

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4 Ridley J H. The histogenesis of endometriosis. A review of facts and fancies. Obstet Gynecol Survey 23 1-35 1968
5 Roland, M Culdoscopy and laparoscopy: Competitive or complementary technics? Fertil Steril 21 361-376, 1970
6 Samuelsson, S. & Sjovall A., On the diagnostic value of laparoscopy in ovarian endometriosis. Acta Obstet Gynecol Scand 47 350-360, 1968
7 Smith B. D & Dillon T F Laparoscopy Fertil Steril 21 193-200 1970.
8. Swolin, K & Rosencrantz, M Laparoscopy a h terosalpingography in sterility investigations. A c parative study Fertil Steril 23 270, 1972.

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Table II. *Gestation period and birth weight of live births in women* [illegible]*ith neuroses and in women reporting no disease*

	Maternal disease		Statistical decision
	Neuroses	None	
Total number of live births	388	112 328	
Number [illegible]ith recorded gestation period	365	108 622	
Mean gestation period, in [illegible]eeks	39 4	39.9	Not sign.
Prematurely-born infants, percentage (Gestation period 37 weeks)	8 8	5 0	p 0 001
Number [illegible]ith recorded birth weight	387	112 000	
Infants of low birth [illegible]ght, percentage (2 500 grams or less)	8 5	3 7	p 0 001
Mean birth weight in grams	3 334 0 (diff. 162 7)	3 496.7	$p<0\ 001$
Gestation-period-adjusted mean birth weight	3 370 9 (diff 109 7)	3 480 6	

At birth, malformation, disease or birth injury were noted in 10.9% of the births of neurotic [illegible]omen, a figure which is *significantly* higher than the 3 9% in controls. A statistically significant difference is also found with respect to congenital malformation *per se*. Down's syndrome malformation of the nervous system and urogenital malformation are particularly preponderant in births amongst neurotic women. Each of these malformations was observed in 3 births, while the figures to be expected—on the basis of the frequencies in births of the controls—are 0.3 with Down's syndrome, 0 6 with nervous system malformation and 0 8 with urogenital malformation.

Data presented in Table II compare live births of neurotic women and of controls with respect to gestation period and birth weight. It can be seen that prematurely-born infants and low-birth-weight infants are more frequent among births of neurotic women. The mean birth weight of infants of the neurotic women is 162.7 grams less than that of infants of the controls. Further analysis of the mean birth weights revealed that the difference is reduced by only 1 7 grams after exclusion of infants of multiple pregnancies and by 53 0 grams (i.e. 33%) after adjustment by gestation period.

Furthermore, hypoxia at birth was significantly more frequent in infants of women with neuroses than in infants of controls (2.3% vs. 0.7%)

A comparison of mortality in the two groups of births is presented in Table III. It can be seen that the stillbirth- perinatal- neonatal- postneonatal- and infant mortality rates are all higher in births among neurotic women than in births among controls. The differences are all statistically significant, except in the case of the stillbirth rate. It is particularly noteworthy that mortality during the first year of life in infants of neurotic women is 3 6 times that in infants of controls. Adjustment of the infant mortality rates by birth weight reduced the difference to about one-third of its original [illegible]alue, but still the rate is 1.8 times higher for infants of neurotic women.

T[illegible]ble III. *Mortality rates of births in women with neuroses and in women reporting no disease*

Reported disease of mother	Total births	Live births	Mortality rates per 1000*				
			Still-birth	Peri-natal	Neo-natal	Post-neonatal	Infant
Neuroses	393	388	10.2 (4)	35.7 (14)	28 4 (11)	12.9 (5)	41.3 (16)
None	113 511	112 328	7.8 (883)	14 6 (1657)	8 0 (898)	3 4 (382)	11 4 (1280)
Statistical decision			Not sign. p 0 05	Significant p 0 001	Significant p 0 001	Significant p 0.005	Significant p 0 001

* Fetal deaths of less than 28 [illegible]eeks of gestation are excluded in calculating the stillbirth and perinatal mortality rates. Figures in parentheses are the actual numbers of deaths.

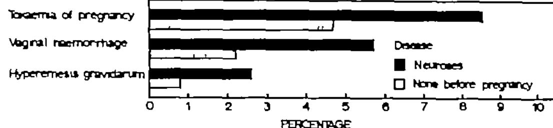


Fig 1 Frequencies of antepartum complications in women with neuroses and in women reporting no disease before pregnancy

The variables studied include complications during pregnancy conduct of labour gestation period, birth weight frequency of malformation, disease or birth injury stillbirths, and infant deaths. In the comparison of complications during pregnancy the controls consist of pregnancies in women reporting no disease before pregnancy a total of 1.5 423 The study of infant mortality rate is made possible through the linkage of the material of the Medical Birth Registry with that of the official registration of deaths from the Central Bureau of Statistics of Norway

The data have been analysed by applying the chi-square test in comparing frequencies and the one-sided *t*-test in comparing two arithmetic means An observed difference is considered statistically significant if p is less than 0.05 In the comparison of certain variables the direct or the indirect method of adjustment has been applied, information on the total birth population of Norway during 1967–68 has been used as the standard.

FINDINGS

Pregnancy and labour

A comparison between the two groups with respect to frequencies of antepartum complications is illustrated in Fig. 1 Neurotic women have toxaemia of pregnancy in 8 5% as against 4 7% in the controls, vaginal haemorrhage in 5 7% as against 2.2% and hyperemesis gravidarum in 2.6% as against 0 8% All these differences are statistically significant.

The percentage of induced labour is significantly higher in the neurosis group (22.7) than in the controls (9 1). Complicated labour is also significantly more frequent in the former group (16 2% vs. 9 6%).

Intervention during labour in neurotic women was resorted to in 18.0% of the births, a figure which is more than double that in controls (7 7%) All main types of intervention, namely artificial rupture of the membranes, forceps or vacuum extraction and caesarean section, are significantly more frequent in women with neuroses (Table 1)

Births

The total number of births of neurotic women was 395 and of controls, 113 511 The percentage of male births in neurotic women was 54 4 which is not significantly different from that in controls (51 1). Multiple birth however occurred in 7 pregnancies in neurotic women, i.e. in 1 8% a figure which is significantly higher than the 0 9% in controls.

Table 1 *Intervention during labour in births in women with neuroses and in women reporting no disease*

Reported disease of mother	Total births	Births with intervention		Rupture of membranes		Forceps of vacuum ext.		Caesarean section		Other	
			%		%		%	n	%		
Neuroses	395	71	18.0	16	4 1	18	4 6	17	4.3	40	10 1
None	113 511	8 709	7 7	1 635	1 4	2 693	2 4	1 262	1 1	4 369	3
Statistical decision		$p<0.001$		$p<0.001$		$p<0.005$		$p<0.001$		p 0.001	

Table II. *Gestation period and birth weight of live births in women with neuroses and in women reporting no disease*

	Maternal disease		Statistical decision
	Neuroses	None	
Total number of live births	388	112 328	
Number with recorded gestation period	365	108 622	
Mean gestation period, in weeks	39.4	39.9	Not sign.
Prematurely-born infants, percentage (Gestation period < 37 weeks)	8.8	5.0	$p < 0.001$
Number with recorded birth weight	387	112 000	
Infants of low birth weight, percentage (2 500 grams or less)	8.5	3.7	$p < 0.001$
Mean birth weight in grams	3 334.0 (diff. 162.7)	3 496.7	$p < 0.001$
Gestation-period-adjusted mean birth weight	3 370.9 (diff. 109.7)	3 480.6	

At birth, malformation, disease or birth injury were noted in 10.9% of the births of neurotic women, a figure which is significantly higher than the 3.9% in controls. A statistically significant difference is also found with respect to congenital malformation *per se*. Down's syndrome, malformation of the nervous system and urogenital malformation are particularly preponderant in births amongst neurotic women. Each of these malformations was observed in 3 births, while the figures to be expected—on the basis of the frequencies in births of the controls—are 0.3 with Down's syndrome, 0.6 with nervous system malformation and 0.8 with urogenital malformation.

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Statistical decision			Not sign. $p > 0.05$	Significant p 0.001	Significant p 0.001	Significant $p < 0.005$	Significant $p < 0.001$

* Fetal deaths of less than 28 weeks of gestation are excluded in calculating the stillbirth and perinatal mortality rates. Figures in parentheses are the actual numbers of deaths.



Fig 1 Frequencies of antepartum complications in women with neuroses and in women reporting no disease before pregnancy

The variables studied include complications during pregnancy conduct of labour gestation period birth weight, frequency of malformation, disease or birth injury still births, and infant deaths. In the comparison of complications during pregnancy the controls consist of pregnancies in women reporting no disease before pregnancy a total of 1.5 423 The study of infant mortality rate is made possible through the linkage of the material of the Medical Birth Registry with that of the official registration of deaths from the Central Burea of Statistics of Norway

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FINDINGS

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Table I *Intervention during labour in births in women with neuroses and in women reporting no disease*

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Statistical decision		$p<0.001$		$p<0\ 001$		$p<0\ 005$		$p<0\ 001$		p 0 001	

REFERENCES

1. Abramson, J H Singh, A R. & Mbambo, V.: Antenatal stress and the baby's development. Arch Dis Childh 36 42, 1961
2. Bakketeig, S. L. & Bjerkedal, T The course and outcome of pregnancy in women with bronchial asthma. Acta Allergol 27 397 1972.
3. Bjerkedal, T & Bakketeig, S. L. The course and outcome of pregnancy in women with epilepsy Acta Obstet Gynaecol Scand 52: 245 1973.
4. Bjerkedal, T & Bakketeig, S L. The course and outcome of pregnancy in women with pulmonary tuberculosis. In manuscript.
5. Bjerkedal, T & Bakketeig, L.: Medical Registration of Births in Norway 1967–68. Some Descriptive and Analytical Aspects. Institute of Hygiene and Social Medicine, University of Bergen, Norway 1972.
6. Blau, A., Slaff, B., Easton, K., Welkowitz, J Springarn, J. & Cohen, J The psychogenic etiology of premature births. A preliminary report. Psychosom Med 25 201, 1963.

Brown, L. Anxiety in pregnancy Br J Med Psychol 37 47 1964

. Caldwell, J Personality in pregnancy and labour South Med J 51 1026, 1958.

Davids, A. & De Vault, S. Maternal anxiety during pregnancy and childbirth abnormalities. Psychosom Med 24 464 1962

Destounis, N Complications of pregnancy: A psychosomatic approach. Canad Psychiat Ass J 7 279 1962.

1. Grimm, E R. Psychological investigation of habitual abortion Psychosom Med 24 369 1962.
2. Gunther L M Psychology and stress in the life experience of mothers of premature infants A comparative study Amer J Obstet Gynecol 86 333, 1963.
3. Harvey W A. & Sherley M. J. Vomiting in pregnancy: A psychiatric study Psychosom Med 16 1, 1954.
4. Hedberg, E Holmdahl, K & Pehrson, S. On relationship between maternal health and congenital malformations. Acta Obstet Gynecol Scand 46 378, 1967
15. Mann, E. C. The role of emotional determinants in habitual abortion. S Clin North America 37 447 1957
16. McDonald, R. L. Personality characteristics in patients with three obstetric complications. Psychosom Med 27 383, 1965.
17. McDonald, R. L. & Christakos, A. C. Relationship of emotional adjustment during pregnancy to obstetric complications. Amer J Obstet Gynecol 86 341, 1963
18. Nuckolls, K. B Cassel, J & Kaplan, B H. Psychological assets, life crisis and the prognosis of pregnancy Amer J Epidemiol 95. 431, 1972.
19. Ringrose, C. A. D Psychosomatic influences in the genesis of toxemia of pregnancy Canad Med Ass J 84 647 1961
20. — Further observations on the psychosomatic character of toxemia of pregnancy Canad Med Ass J 84 1064, 1961
21. Rosen, S.: Emotional factors in nausea and vomiting of pregnancy Psychiat Quart 29 621 1955
22. Scott, E. M. & Thompson, A. M A psychological investigation of primigravidae. IV Psychological factors and the clinical phenomena of labour J Obstet Gynaecol Br Emp 63 502, 1956.
23. Scott, D H. Some psychosomatic aspects of casualty in reproduction J Psychosomatic Res 3 42, 1958
24. Sontag, L. W Effect of fetal activity on nutritional state of infant at birth. Amer J Dis Child 60 621 1940
25. — The significance of fetal environmental differences. Amer J Obstet Gynecol 42 996, 1941
26. World Health Organization: International Classification of Diseases. 1965 Revision, vol. 1 p. 146 Geneva, 1967

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DISCUSSION

As mentioned in "Material and Methods a woman is considered neurotic" if she is recorded to be suffering from a condition classifiable as neurosis. Information given on the registration forms is in most instances insufficient to allow the distinction between those women who are under psychiatric care and those who are not. However the recording of the condition indicates that it is of a sufficient degree to make the woman report it and the attending midwife or physician to record it.

It appears from the findings of this study that women who report neuroses experience pregnancy complications and labour difficulties more often than those who report no disease and that their births have higher rates of morbidity and mortality

In interpreting these findings it is realized that other factors known to influence the course and outcome of pregnancy have not been eliminated by the use of matched controls or by other sampling techniques. It was noted that the neurosis group did not differ from the controls except in being somewhat older However a difference of 2.6 years in the mean age is too small to explain the higher frequency of complications in women with neuroses.

That pregnancy in women with neuroses is more frequently complicated by toxaemia bleeding and hyperemesis, corresponds with reports that anxiety is more common in pregnant women who later develop hyperemesis (7 8 13 21) or toxaemia (16 19 20). Although it seems that anxiety may unfavourably affect pregnancy the underlying mechanism is not established.

That induction of labour and intervention during labour are more frequent in women with neuroses, coincides well with the high frequency of antepartum complications and tends to explain the high frequency of complicated labour Moreover the attendant may be inclined to intervene in order to terminate labour in neurotic women

Frequency of malformation, disease or birth injury is significantly higher in births of neurotic women than in births of controls. Congenital malformations *per se* were twice as frequent and the excess in malformations was particularly noted with respect to Down s syndrome, nervous system malformation and urogenital malformation. This finding agrees with that reported b Scott (23) and contradicts the general conclu of Hedberg et al. (14), who found no signific relation between maternal disease and the o currence of congenital malformations.

The finding that low birth weight is more fr quent in infants of neurotic women is obser also by Gunther (12) but not by others (1 1 Exclusion of multiple births in the present m terial did not affect the difference between t mean birth weights. Adjustment by gestation p riod reduced the difference by only one-th indicating that the lower mean birth weight infants of neurotic women can be only partly plained by the higher frequency of prematu labour As to other possible causes it may noted that Sontag has found that maternal a iety results in fetal hyperactivity (25), and t as the fetal activity increased, the birth wei tended to decrease (24).

The high mortality of births of neurotic wom is an expected consequence of the high f quencies of complicated labour low birth wei and morbid conditions of the infants. It is interest to note that infant mortality is high only in the neonatal- but also in the postn natal period. Since causes of death in the p neonatal period more often are environmen than biological this finding suggests that infant women suffering from neuroses are cared fo an environment—psychological social or bot that is less favourable than that of other infa

The findings of the present study undoubt indicate a need for comprehensive care for pr nant neurotic women. Although the mechani underlying the relation between neuroses and stetric complications are yet to be establis ante- and post natal psychotherapy in addi to efficient obstetric care may be expected lower the complications in neurotic women their newborns. This opinion is supported Destounis (10), who found a lower frequency pregnancy complications in 10 patients recei psychotherapy than in controls.

ACKNOWLEDGEMENTS

The data processing has been performed with the assistance of the EDP-Section of the Medical Faculty Faculty of Bergen. One of us (S. L. B.) is grateful to Norwegian Agency for International Development for post-doctoral fellowship.

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DIAZEPAM IN EARLY HUMAN PREGNANCY

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Abstract. The transfer of diazepam and N-demethyldiazepam across the placenta in early human pregnancy is studied in a group of 12 patients after a single dose, and in a group of 7 patients after the continued use of diazepam for up to a month. After a single dose the feto-maternal ratio of the diazepam concentrations is 1.2, while after continued use it is 0.4. The main reason for this difference is probably the incomplete distribution of diazepam after a single dose at the time the samples are taken. The transfer of N-demethyldiazepam across the placenta occurs just as easily and the feto-maternal ratio after continued treatment is 0.4 too. The concentration of N-demethyldiazepam in the fetal liver is higher than any other concentration measured and this could constitute indirect evidence of metabolism in the fetal liver. The concentrations of diazepam and N-demethyldiazepam in fetal tissues are at a level comparable to the concentration of diazepam that has a damaging effect on cells in cellular cultures.

The use of drugs during the first trimester of pregnancy should be generally avoided if possible. Since diazepam is one of the most common drugs in the world, it is, however possible that a woman takes it without being aware that she has an early pregnancy. Diazepam is also given for the psychiatric complications of pregnancy and is recommended for treating a threatened abortion either for its psychological effect (6) or as an tocolytic agent (1).

Diazepam is considered to be free from any teratogenic effects. There are, however some studies of the damaging effect of diazepam in cell cultures. Thus, in an electronmicroscopical study Breen & Stenchever have noticed some alterations ... lture of human fibroblasts wh... added in different concentratio... concentration of 1 μg/ml of diazep... ugh to cause deformation in the membranous elements of the cells. It was also demonstrated that diazepam is capable of producing chromosome breakage in vitro and in vivo and that it is capable of retarding the growth rate of the cells (11, 12). Although not all studies are in agreement with these results (3, 9, 10) they must be kept in mind when diazepam is considered for use during early pregnancy.

Our previous studies show that diazepam and its main metabolite, N-demethyldiazepam, easily cross the placenta when diazepam is given to the mother during labour (4, 5). Besides this the concentrations of diazepam and N-demethyldiazepam in the newborn are significantly higher than in the mother. Idänpään-Heikkilä et al. have noticed in their study with C[14]-labelled diazepam that diazepam crosses the placenta very early in pregnancy (7). They also found a higher concentration of diazepam in the fetus than in the mother.

According to the current view, passive diffusion never takes place against the concentration gradient. It is therefore curious that diazepam concentrations can be higher in the fetus than in the mother. The previous studies were made with single doses, and the samples were collected at such an early stage that the distribution of diazepam may have been still incomplete and a steady state not yet reached. Because diazepam is known to accumulate in the tissues and to reach a steady state in about 4 days (13), we have compared the concentrations of diazepam in the mother and fetus after a single dose, and after continued use, and studied whether the concentrations of diazepam and N-demethyldiazepam

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DIAZEPAM IN EARLY HUMAN PREGNANCY

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Abstract The transfer of diazepam and *N*-demethyldiazepam across the placenta in early human pregnancy studied in group of 12 patients after single dose, and in group of 7 patients after the continued use of diazepam for up to month. After single dose the feto-maternal ratio of the diazepam concentrations is 1 2, but after continued use it is 0.4 The main reason for this difference is probably the incomplete distribution of diazepam after single dose at the time the samples are taken The transfer of *N*-demethyldiazepam across the placenta occurs just as easily and the feto-maternal ratio after continued treatment is 0 4, too The concentration of *N*-demethyldiazepam in the *fetal liver is higher* than any other concentration measured and this could constitute indirect evidence of metabolism in the fetal liver The concentrations of diazepam and *N*-demethyldiazepam in fetal tissues are at level comparable to the concentration of diazepam that has damaging effect on cells in cellular cultures.

The use of drugs during the first trimester of pregnancy should be generally avoided if possible Since diazepam is one of the most common drugs in the world, it is, however possible that a woman takes it without being ware that she has early pregnancy Diazepam is also given for the psychiatric complications of pregnancy and is recommended for treating a threatened abortion either for its psychological effect (6) or as an tocolytic agent (1).

Diazepam is considered to be free from any teratogenic effects. There are, however some studies of the damaging effect of diazepam in cell cultures. Thus, in an electronmicroscopical study Breen & Stenchever have noticed some lterations in the cell culture of human fibroblasts when diazepam was added in different concentrations (2). E en a concentration of 1 $\mu g/ml$ of diazepam was high enough to cause deformation in the membranous elements of the cells. It was also demonstrated that diazepam is capable of producing chromosome breakage in vitro and in vivo and that it is capable of retarding the growth rate of the cells (11 12). Although not all studies are in agreement with these results (3 9 10) they must be kept in mind when diazepam is considered for use during early pregnancy

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Table I *The age and weight of the patients the duration of the pregnancy and the duration of the treatment with diazepam*

Patient	Age (years)	Weight (kg)	Duration of pregnancy (weeks)	Duration of treatment (days)
S. K.	30	72	14	9
B. L.	25	59	14	15
S. L.	35	102	14	16
U V	31	78	12	17
K. T	43	54	14	23
R. J	46	97	15	24
E. S.	34	59	14	30

in the fetal tissues are comparable to the concentrations that have a damaging effect in the cell cultures.

MATERIAL AND METHOD

Twelve patients (mean age 3_5 years, mean weight 69 6 kg) admitted to the hospital for legal abortion by hysterotomy received 10 mg diazepam (Tensopam® Lääke Oy) and 0.5 mg atropine intramuscularly as preoperative medication. Anesthesia was induced with thiopentone up to 350 mg intravenously and maintained with a 70/30% nitrous oxide/oxygen mixture Relaxation was induced with 30 mg tubocurarine and continued with succinyl-choline infusion During the operation pethidine or Thalamonal® were given as requested. The fetuses (from 12 to 15 weeks) were removed 40–120 minutes after premedication. At the same time as the removal of the fetuses, a blood sample was drawn from the ante-cubital vein of the mother and 3 minutes later a blood sample was taken from the umbilical cord.

Seven patients received diazepam (Tensopam® Lääke Oy) 5 mg 3 times daily by mouth for psychiatric reasons, or voluntarily before the hysterotomy The age and weight of these patients, as well as the duration of treatment with diazepam and the duration of pregnancy are shown in Table I. The last dose of diazepam was given on the evening before the operation. 0 3 mg of scopolamine combined with 10 mg of morphine or 0.5 mg of atropine combined with 50 mg of pethidine were given as premedication. Anesthesia was performed and blood samples collected as described above After 10 minutes the tissue samples from the placenta, fetal brain and fetal liver were taken. In one case when the fetus was removed within the amniotic sac, a sample of amniotic fluid was also taken.

The blood samples were centrifuged and the plasma separated and stored at +4°C until analysed The tissue samples were quick-frozen and thawed just before homogenisation. The sample of amniotic fluid was handled in the same way as the blood samples. The concentrations of diazepam and N-demethyldiazepam were determined with a gas chromatograph with and Ni[63]-electroncapture detection as described in an earlier paper (4). The recovery percentage of diazepam standard from the plasma and amniotic fluid is 94 and from the tissues, 85 The corresponding recovery percentages of N-demethyldiazepam standards are 9_ and 90 The precision of double determinations, which were always performed, was 0.5%.

RESULTS

The concentrations of diazepam after a 10 mg intramuscular injection as a single dose are shown in Table II. The difference between the fetal and maternal concentration is not significant. The mean feto-maternal ratio of the individual case pairs is 1 2. Small amounts of N-demethyldiazepam were found in only three samples of maternal plasma.

The individual concentrations of diazepam and *N*-demethyldiazepam after continued use are shown in Table III and the mean concentrations are shown in Fig. 1 The difference between diazepam concentration in fetal and maternal plasma is highly significant ($p < 0.001$). The feto-maternal ratio of the diazepam concentrations is 0 4 In one sample of amniotic fluid the concentration of diazepam was _8 ng/ml.

The difference between the concentrations of N-demethyldiazepam in fetal and maternal plasma is very significant ($p < 0.01$). The feto-maternal ratio of concentrations of N-demethyldiazepam is 0 4 The difference between the concentrations of *N*-demethyldiazepam in fetal plasma and liver is significant ($p < 0.05$).

Table II *Plasma concentration of diazepam in mother and fetus following an intramuscular injection of 10 mg of diazepam*

n = 12	ng/ml ± S.E.M.
Mother	80 ± 13
Fetus	101 ± 32

DISCUSSION

Like Idänpään Heikkilä et al. (7), we found that diazepam easily crosses the placenta at the end of the first trimester of pregnancy The concentration after a 10 mg dose of diazepam is quite comparable to the concentrations noticed by them after a 5 mg dose. We have also found the diaze-

Table III. *Individual concentrations (ng/ml) of diazepam (D) and N-demethyldiazepam (ND) in maternal and fetal plasma and in fetal tissues after continued treatment of the mother with diazepam*

Patient	Maternal plasma		Fetal plasma		Fetal liver		Fetal brain		Placenta	
	D	ND	D	ND	D	ND	D	ND	D	ND
S. K.	207	170	50	37	222	195	236	55	235	120
B. L.	147	288	36	180	109	550	88	163	109	207
S. L.	117	179	45	82	107	280	64	57	107	103
U. V.	157	187	36	54	54	250	28	125	33	113
K. T.	143	351	43	101	88	114	53	64	43	102
R. I.	126	241	36	110	163	234	52	127	61	163
E. S.	240	460	108	277	—	493	—	175	—	200

pam concentration in the fetus to be higher than in the mother.

According to Villee (14), one should conclude that there is active transport if the transfer across a biological membrane occurs against the concentration gradient, provided that the concentrations have reached a steady state. After a single dose the distribution of the drug may be incomplete and the higher concentration in the fetus only apparent at the moment the samples are taken. Idanpaan-Heikkila et al. suggested the possibility of a higher affinity of the fetal red cells or plasma proteins for diazepam (7).

We have noticed, however, that the concentration in the fetal plasma is higher than in the mother and thus a higher affinity for fetal red cells seems to be unlikely. When, on the other hand, 96% of diazepam is bound with plasma proteins in adults (13), it is hard to believe that the binding with fetal plasma proteins is higher. A possible explanation for the higher concentration in the fetal plasma is incomplete distribution, which would be in agreement with the general view of the penetration of drugs through the placenta.

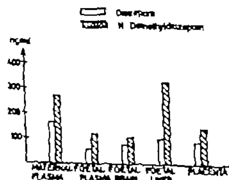


Fig. 1. The mean concentrations of diazepam and N-demethyldiazepam in maternal and fetal plasma and in fetal tissues after continued use of diazepam.

The concentrations of diazepam and *N*-demethyldiazepam in the mother are somewhat low considering the dose. Van der Kleijn et al. noticed that the concentration of diazepam was about 800–1 000 ng/ml in plasma, when the dose was 10 µg three times daily by mouth over 15 days (13). The concentration of *N*-demethyldiazepam was 300–600 ng/ml and in some cases still increased even after 2 weeks. We cannot be sure that the patients had taken the drug regularly which may be the reason for the low concentrations. The plasma concentration of *N*-demethyldiazepam, however, rises steadily with the duration of the treatment and the weight of the patients.

We believe that if the patients had used a higher dose of diazepam, for example 10 mg 4 times daily, which is recommended in treating threatened abortion (6) much higher concentrations of diazepam and *N*-demethyldiazepam would have been found both in the plasma and in the tissues.

The tissue concentrations we have found are in some cases, however, high enough to be comparable to the concentrations that have a damaging effect on cell cultures. It is of course, not absolutely certain that the alterations described in cell cultures also take place in vivo, when the effect of the drug is modified by many homeostatic mechanisms. All cellular damage produced by diazepam in vitro could, however, be reversible in vivo. It is also possible that all alterations are only evidence of cellular metabolism and are thus harmless.

The concentration of *N*-demethyldiazepam in

the fetal liver is exceptionally high and may be indirect evidence of the metabolism in the liver cells at this stage of pregnancy. A similar accumulation of diazepam in the liver was noticed earlier in animal experiments (8).

As a conclusion we again emphasize the importance of avoiding the prescription of drugs to pregnant patients if the need for treatment is not urgent. Minor tranquillizers, particularly are seldom absolutely indicated during pregnancy. Although diazepam is considered a harmless drug the concentrations noticed in the fetus are high enough to be pharmacologically active in adults, too, and thus diazepam may have an effect on the developing fetus.

REFERENCES

1. Boden, W. Tokographische Untersuchungen über den Einfluss von Valium bei drohender Frühgeburt. Fortschr Geburtsh Gynakol *19* 356, 1964
2. Breen, P. C. & Stenchever, M. A. Some effects of diazepam on the fine structure of human fibroblasts in tissue culture. Am J Obstet Gynecol *108* 520, 1970
3. *Cohen, M. M. Hirschhorn, K. & Frosch, W. A.* Cytogenetic effects of tranquilizing drugs in vivo and in vitro. J Amer Med Ass ?07 425 1969
4. Erkkola, R. Kangas, L. & Pekkarinen, A. The transfer of diazepam across the placenta during labour. Acta Obstet *Gynecol Scand 52. 167 1973*
5. Erkkola, R. & Kanto, J. Concentration levels of diazepam and its main metabolite, *N*-demethyldiazepam, in the circulatory system of mother and child during the delivery. Proceed in 3rd Europ Congr Perinat Med, Lausanne April 1972.
6. Höter, J. Über Wirksamkeit, Nebenwirkungen, I[illegible]dikationen und Kontraindikationen von D[illegible] (Valium) in der Geburtshilfe und Frauenheilkunde. Klin Wschr *46* 681 1968
7. Idänpään-Heikkilä, J. E., Jouppila, P. I., Puolakka, J. O. & Vorne, M. S. Placental transfer and fe[illegible] metabolism of diazepam in early human pregnancy. Am J Obstet Gynecol *109* 1011 1971
8. Idänpään-Heikkilä, J. E., Taska, R. J., Allen, H. A. & Schoolar, J. G. Placental transfer of diazepam-¹⁴C in mice, hamsters and monkeys. J Pharmacol Exp Ther *176* 752, 1971
9. Schmid, W. & Staiger, G. R. Chromosome studies on bone marrow from chinese hamsters treated with benzodiazepine tranquillizers and cyclophosphamide. Mutat Res *7* 99 1969
10. Staiger, G. R.: Chlordiazepoxide and diazepam: absence of effects on the chromosomes of diploid human fibroblast cells. Mutat Res *7* 109 1969
11. Stenchever, M. A. & Frankel, R. B. Some effects of diazepam in human cells in vitro. Am J Obstet Gynecol *103* 836, 1969
12. Stenchever, M. A., Frankel, R. S. & Jarvis, J. A. Effect of diazepam on chromosomes of human leucocytes in vivo. Am J Obstet Gynecol *107* 456 1970.
13. Van der Kleijn, E. van Rossum, J. M. Muskens, E. T. J. M. & Rijntjes, N. V. M.: Pharmacokinetics of diazepam in dogs, mice and humans. Acta Pharmacol Toxicol *29* 109 1971
14. *Villee, C. Placental transfer of drugs.* Ann N Y Acad Sci *123* 237 1965

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ULTRASTRUCTURAL SIGNS OF AN INTERFERENCE IN THE CARBOHYDRATE METABOLISM OF HUMAN ENDOMETRIUM PRODUCED BY THE INTRAUTERINE COPPER T DEVICE

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Abstract Biopsies were obtained from 4 women on cycle days 10–12 and 20–23 before the insertion of copper T device and on the same stages several times during one year with the device in place. Further control biopsies ere obtained on the same cycle days from 3 cases having a Tatum-T device fitted copper. The biopsies were processed for light and electron microscopy of both glandular and luminal uterine epithelium. The copper-influenced endometria differed from the controls by lacking light areas in the cytoplasm. The light areas are associated with carbohydrate metabolism and their lacking might indicate an impaired degradation of glycogen. Thus, the secretory activity of the epithelium can be faulty resulting in an interference in the viability of the blastocyst

The addition of copper wire to small T-shaped intrauterine device (Cu-T) has greatly improved the contraceptive effect of this device (33). A device with a copper area of 120–200 m released 30–50 µg of copper per 24 hours and increased the copper concentration of the endometrium and cervical mucus (5)

Animal experiments concerning the mode of action of the device have been performed by several investigators and a review of these data has been published by T tum (26). The mechanism of



Fig 1 Endometrium in secretory phase obtained from control biopsy. The luminal epithelium and cross-sectioned gland is seen. Glycogen appears darkly stained and is observed in the gland cells but hardly in the luminal cells. The lower content of glycogen is one factor distinguishing the luminal epithelium from the glandular one 350

the fetal liver is exceptionally high and may be indirect evidence of the metabolism in the liver cells at this stage of pregnancy A similar accumulation of diazepam in the liver was noticed earlier in animal experiments (8).

As a conclusion we again emphasize the importance of avoiding the prescription of drugs to pregnant patients if the need for treatment is not urgent. Minor tranquillizers, particularly are seldom absolutely indicated during pregnancy Although diazepam is considered a harmless drug the concentrations noticed in the fetus are high enough to be pharmacologically active in adults, too, and thus diazepam may have an effect on the developing fetus.

REFERENCES

1 Boden, W Tokographische Untersuchungen uber den Einfluss von Valium bei drohender Frühgeburt. Fortschr Geburtsh Gynäkol *19* 356, 1964
2 Breen, P C. & Stenchever M A. Some effects of diazepam on the fine structure of human fibroblasts in tissue culture Am J Obstet Gynecol *108* 520 1970
3 Cohen M M Hirschhorn, K. & Frosch, W A. Cytogenetic effects of tranquilizing drugs in vivo and in vitro. J Amer Med Ass *207* 2425 1969
4 Erkkola R., Kangas, L. & Pekkarinen, A The transfer of diazepam across the placenta during labour *Acta Obstet Gynecol Scand 52 167 1973*
5 Erkkola, R. & Kanto, J Concentration levels of diazepam and its main metabolite *N*-demethyldiazepam, in the circulatory system of mother and child during the delivery Proceed in 3rd Europ Congr Perinat Med, Lausanne April 1972.
6. Hüter J Über Wirksamkeit, Nebenwirkungen, Indikationen und Kontraindikationen von Diazepam (Valium) in der Geburtshilfe und Frauenheilkunde Klin Wschr 46. 681 1968
7 Idänpään Heikkilä, J E. Jouppila, P I Puolakka, J O & Vorne, M S. Placental transfer and fetal metabolism of diazepam in early human pregnancy Am J Obstet Gynecol *109* 1011 1971
8 Idänpään-Heikkilä J E, Taska, R. J Allen, H. A & Schoolar J C Placental transfer of diazepam-¹⁴C in mice, hamsters and monkeys. *J Pharmacol Exp Ther 176*. 752, 1971
9 Schmid, W & Staiger G R. Chromosome studies on bone marrow from chinese hamsters treated with benzodiazepine tranquillizers and cyclophosphamide Mutat Res 7 99 1969
10. Staiger G R. Chlordiazepoxide and diazepam. *Absence of effects on the chromosomes of diploid* human fibroblast cells. Mutat Res 7 109 1969
11. Stenchever M A. & Frankel, R. B. Some effects of diazepam in human cells in vitro. Am J Obstet Gynecol *103* 836, 1969
12. Stenchever M A. Frankel, R. S. & Jarvis, J A Effect of diazepam on chromosomes of human leucocytes in vivo Am J Obstet Gynecol *107* 456, 1970.
13 Van der Kleijn, E., van Rossum J M Muskens, E. T J M & Rijntjes, N V M Pharmacokinetics of diazepam in dogs, mice and humans. Acta Pharmacol Toxicol *29* 109 1971
14 Villee C., Placental transfer of drugs. Ann N Y Acad Sci *123* 237 1965

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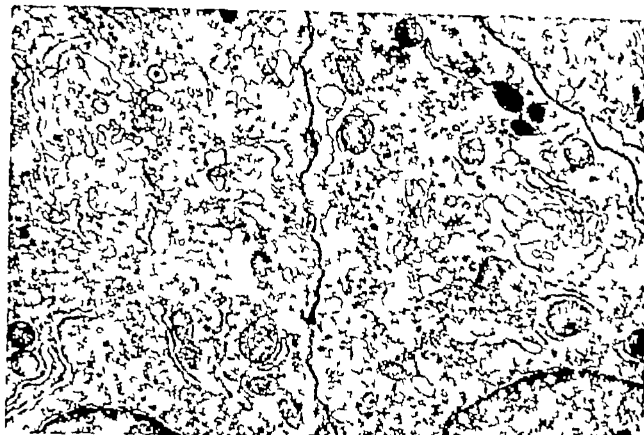


Fig 2 Middle parts of glandular epithelium in proliferative phase obtained from a control biopsy The Golgi apparatus is moderately well developed. Dense granules and multivesicular bodies are present in its surrounding × 20 000

action of the Cu-T device in women is unknown

Morphological studies using light microscopy did not demonstrate any marked changes in the endometrium of women using the Cu-T device (7) but preliminary results of the present ultra structural study suggested an interference with the carbohydrate metabolism (17) Also scanning electron microscopy revealed changes, probably of a similar origin, in endometrial surfaces influenced by the device (18). Furthermore the intrauterine presence of the Cu T device induced significant changes in the activity of several endometrial enzymes (6, 12, 21)

The purpose of the present paper is to report the type and possible significance of the changes which were found by electron microscopy in the human uterine epithelium when it was subjected to the influence of an intrauterine device containing copper

MATERIAL AND METHODS

The intrauterine device used in the present study was the Tatum T device with 120, 135 or 200 m copper area. The endometrial biopsies were obtained simultaneously with those used for light microscopical studies and for trace element analyses (5–7). Specimens were obtained from 4 women on cycle days 10–1 and 20–2 on several occasions during one year with the device in situ as well as in control cycles before the insertion of the device and on the same cycle days of the first and second cycle following its removal. Furthermore in 3 women a Tatum-T device without copper was inserted for a period of 5–9 months and several biopsies were obtained on the same cycle days a bove with the device in situ. The endometrial biopsies were obtained with a Novak or Randall type suction curett without anesthesia and without dilation of the cervix.

The biopsies were fixed immediately by immersion in 5% solution of glutaraldehyde in Soerensen's phosphate buffer pH 7 and were kept in the fixative for periods of one day up to several months At embedding small specimens were cut from the biopsies, rinsed in the buffer and post-fixed for 3 hours in a solution of 1 osmium tetroxide in Soerensen phosphate buffer

Fig 3 Apical parts of glandular epithelium in proliferative phase obtained from a control biopsy Several smooth-surface vesicles are observed. Glycogen granules of the β-type are numerous in the supranuclear part of the cytoplasm in this section 1 000

lum were frequent in the apical parts of some ells (Fig. 3). In the luteal phase on cycle days 0–22, the cell surface was bulging and often resented irregular projections (Fig. 4). The rough ndoplasmic *reticulum* occurred more extensively and the smooth surfaced vesicles were frequent (Fig. 5). The cytoplasm displayed many glycogen granules, both of the α and β-type. Small vacuoles with a diameter less than 1 μm were present inside the groups of glycogen granules. Larger vacuole-like structures, here called light areas, having a *maximum size of* about 6 μm seemed to be structurally related to the glycogen granules (Fig. 4). These light areas were observed in all parts of the cells. Dense granules and glycogenosomes were noticed, and in the basal part f the cells giant mitochondria were present.

The luminal epithelium displayed less pronounced *intracellular changes during* the cycle. t was lower and had fewer and less developed signs of glycogen turn-over (glycogen granules, light areas, smooth vesicles and glycogenosomes). Only occasionally did giant mitochondria appear during the luteal phase.

Control specimens obtained from women after removal of the Cu-T device

The glandular and luminal epithelium had an *appearance similar to the epithelia of the* specimens obtained before the insertion of the device.

Control specimens obtained from women using the T device without copper

Light and electron microscopic examination of biopsy specimens obtained from women using a T device without copper *did not differ from* those obtained in women prior to the insertion of the device

Specimens obtained from women with the Cu-T device

The glandular and luminal epithelium in the specimens obtained on cycle days 10–12 did not differ in structure from those obtained at the same stage in the cycle prior to the insertion of the device (Fig. 6). Even some light areas were occasionally noticed (Fig. 7).

The glandular epithelium obtained on days 20–23 displayed an intracellular architecture where several of the features observed in the control specimens were recognized. Thus, dense granules,



Fig. 5. Cross-sectioned apical protrusions of glandular epithelium in secretory phase obtained from control biopsy. The protrusions contain glycogen granules and smooth-surfaced vesicles probably belonging to the endoplasmic reticulum. 16 000

glycogen granules of the α- and β-type, glycogen vacuoles, smooth vesicles, glycogenosomes and giant mitochondria were seen (Figs. 8–9). However the light areas were less frequent and when present, they occupied a smaller area of the cytoplasm than in the control specimens. This difference *in structure* between *normal* glands and glands influenced by the Cu-T device was obvious, but the design of the present experiment does not exclude that other less evident, changes might have occurred at the same time.

The luminal epithelium appeared to be rather similar to that of the control specimens (Fig. 10). *No light areas were noticed but these were few* also in the control specimens.

DISCUSSION

Since only a few biopsies were available from women using a T-device without copper most of the control specimens in the present study were obtained from women prior to the insertion of the Cu-T device. The plain T" did not cause ultrastructural changes in the 3 women tested and it seemed as if the changes observed



Fig. 4. Apical parts of glandular epithelium in secretory phase obtained from a control biopsy. Various types of cellular protrusions are visible to the left and right protrusions filled with glycogen granules, and in the middle two protrusions containing light areas. 16 000.

pH 7.2. Dehydration was performed in ethanol, and the specimens were embedded in Epon.

For light microscopy the sections were stained in a basic solution of Toluidine Blue. Appropriate areas of glandular or luminal epithelium were selected and trimmed for sectioning for electron microscopy.

For transmission electron microscopy the sections were stained with uranyl acetate followed by lead citrate.

RESULTS

Control specimens obtained from women before insertion of the Cu-T device

The epithelial cells of the human endometrium occur as glandular epithelial cells localized in the uterine glands and as luminal epithelial cells covering the uterine surface (Fig. 1).

The glandular epithelium of the control specimens did not differ in appearance from that which has been reported for normal human endometrium (2, 15–16, 22–23, 25, 29, 31–32 and others). The epithelium obtained on cycle days 10–12 displayed a moderate amount of rough endoplasmic reticulum, some multivesicular bodies, irregularly scattered glycogen granules, mostly of the β-type and a rather large Golgi apparatus with dense granules (Figs. 2–3). Vesicles, probably of the smooth endoplasmic retic

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The glandular epithelium obtained on days 20- 23 displayed an intracellular architecture where several of the features observed in the control specimens were recognized. Thus, dense granules,



Fig. 5. Cross-sectioned apical protrusions of glandular epithelium in secretory phase obtained from control biopsy. The protrusions contain glycogen granules and smooth-surfaced vesicles probably belonging to the endo- plasmic reticulum. 16 000

glycogen granules of the α- and β-type, glycogen vacuoles, smooth esicles, glycogenosomes and giant mitochondria were seen (Figs. 8–9). How ever the light areas were less frequent and when present, they occupied a smaller area of the cyto- plasm than in the control specimens. This differ ence in structure between normal glands and glands influenced by the Cu T device was ob- vious, but the design of the present experiment does not exclude that other less evident, changes might have occurred at the same time.

The luminal epithelium appeared to be rather similar to that of the control specimens (Fig. 10). No light areas were noticed but these were few also in the control specimens

DISCUSSION

Since only few biopsies were available from women using a T-device without copper most of the control specimens in the present study were obtained from women prior to the inser tion of the Cu-T device. The plain T" did not cause ultrastructural changes in the 3 women tested and it seemed as if the changes observed



Fig 6. Basal parts of glandular epithelium in prolifera tive phase obtained from a biopsy of copper-influenced endometrium. Membranes of the rough endoplasmic reticulum are visible and an early giant mitochondrion is observed. Glycogen granules are scattered in the cytoplasm. × 20 000

in biopsies from Cu T users were elicited by the copper component of the device and not by the device *per se* Since the biopsies obtained from women *after* the removal of the Cu T device were similar in ultrastructure to those obtained before the insertion, the action observed of the device on the uterine epithelium was reversible This is in agreement with the clinical observa tions of rapidly restored fertility after discontinuation of the method.

The present study comprised standard electron microscopy and no attempts were made to evaluate the findings quantitatively Thus changes additional to what is reported in this paper can not be excluded. The differences observed—a lack of light areas in the Cu-T specimens—are seen in the glandular epithelium As far as the light microscopical dating of the biopsies indicates (Dr Elisabeth Johannisson) the difference should not depend upon any retarded uterine development Rather the difference observed suggests an interference with the carbohydrate metabolism. Since some recent biochemical reports point to a similar effect of the Cu T device the glycogen metabolism of the uterine epithelium will be discussed from an ultrastructural point of view

The various products of glycogen metabolism are to a certain extent dissolved during the pre-



g 7 Middle parts of glandular epithelium in prolifera- ra phase obtained from biopsy of copper-influenced idometrium. Glycogen granules are scattered in the cyto- plasm. Light areas are observed among the glycogen granules in the cell to the right. 17 000

aratory steps and are therefore not visualized with the electron microscope. Furthermore, most of the enzymes involved in glycogen metabolism appear in the cytoplasm or are attached to the glycogen molecules and cannot easily be observed with the electron microscope (e.g. 27 30) It is also know that both synthesis and degradation of glycogen occurs during the late luteal phase (4) These facts render a detailed analysis of the ultrastructure of glycogen metabolism difficult. However from an ultrastructural point of view the glycogen granules generally are related to glycogen vacuoles, to profiles of smooth membrane, to light areas, and to lysosome-like bodies.

The glycogen granules of the uterine epithelium are present mostly as β-granules although α-granules (3) are also observed. The α-granules are regarded as polymerized complexes of β-granules. Glycogen vacuoles are usually found inside large groups of glycogen granules, but the significance of the vacuoles is unknown. However both the α and β-granules and the glycogen vacuoles were found in endometria under influence of the Cu-T device.

The profiles of smooth membranes probably belong to the smooth endoplasmic reticulum (SER) and are most frequent in the apical portion of the cells in the luteal phase, often being associated with glycogen granules and/or light areas. Regarding the location of the membranes and the fact that they carry the enzyme glucose-6-phosphatase (10–11 23) it is reasonable to as-

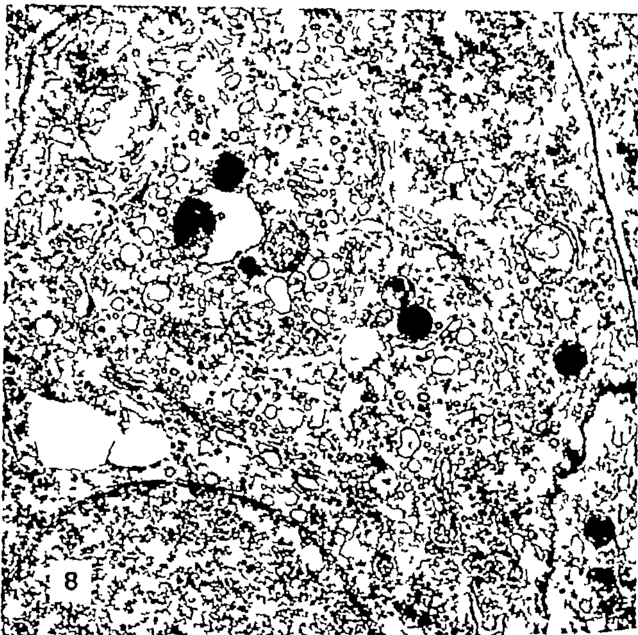


Fig 8. Middle part of a glandular epithelial cell in secretory phase obtained from a biopsy of a copper-influenced endometrium. The Golgi apparatus is well developed. Various types of vesicles and dense bodies are situated in its vicinity × 20 000

sume that they participate in the mechanism of carbohydrate secretion by the epithelium. Although the Cu T device did not alter the appearance of the SER, the activity of the membrane bound enzymes might be affected.

The light areas vary in size the largest areas are mostly devoid of glycogen granules but may contain organelles like mitochondria. These areas give an impression of an artefactual dissolution of the glycogen or an unsuccessful staining of the particles. However the technique used in the present investigation is known to minimize these risks (13) A similar picture can be obtained when liver cells are digested with α-amylase (70) It therefore seems as if the areas represent some step in the hyaloplasmic degradation of glycogen where soluble products are produced Since the presence of a Cu-T device nearly abolished these areas, the device might impair this type of degradation of glycogen

The lysosome-like bodies contain glycogen granules. They are frequent in liver cells and have been named glycogenosomes when filled with glycogen granules (19 23) The glycogeno-



Fig. 9. Apical parts of glandular epithelium in secretory phase obtained from biopsy of copper-influenced endometrium. Many smooth-surfaced vesicles and a group of glycogen granules are visible. × 20 000.

somes are autophagic and are believed to contribute lysosomal degradation of glycogen. The active enzyme might be an α-glycosidase (9). Since these lysosomes, although few in number were present both in copper influenced and normal epithelia, the degradation of glycogen by lysosomes seems to be uninfluenced by the presence of the Cu-T device.

To summarize, the biopsies obtained from women using the Cu-T device lacked most of the large light areas, but displayed both the α and the β-granules, the smooth vesicles and the glycogenosomes. The lack of light areas might, according to the discussion above indicate an interference with the degradation of glycogen by an inhibition of some enzymes. This assumption is supported by the finding that the insertion of a Cu-T device results in a decrease of the α-amylase activity of the endometrium (21). Furthermore, an increase in the endometrial content of glycogen in specimens obtained during the secretory phase seems to follow the use of the Cu-T device (12). Thus, the lack of light areas in the copper influenced endometrium could be associated with an impaired degradation of glycogen and a decreased or faulty secretion of carbohydrates resulting in an accumulation of glycogen in the cells.

The reported findings are of interest with regard to the mode of action of the Cu-T device, as impaired glycogen degradation and/or secretion by the epithelium at the time of implantation might affect the survival of the blastocyst (8, 23). However the effect of copper on carbohydrate metabolism might represent only one of several contraceptive mechanisms. For instance copper seems to affect both the development of the blastocyst (1, 14) and the motility of the sperm cells (28).

ACKNOWLEDGEMENTS

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Fig 10 Middle parts of luminal epithelium in secretory phase obtained from a biopsy of a copper-influenced endometrium. Glycogen granules of both the α- and β-type are visible. In the lower part of the picture the granules are grouped in a body-like structure. × 15 000

REFERENCES

1 Brinster R L. & Cross, P C Effect of copper on the preimplantation mouse embryo Nature 38 398 1972.

2 Cavazos, F Green, J A Hall, D G & Lucas, F V Ultrastructure of the human endometrial glandular cell during the menstrual cycle. Am J Obstet Gynecol 99 833 1967

3 Drochmans, P Morphologie du glycogène Etude au microscope de colorations négati es du glycogène particulaire J Ultrastruct Res 6. 141 1962

4 Hackl, H Zur hormonellen Steuerung der Glykogen-synthese im humanen Endometrium. Arch Gynäk 11 617 1971

5 Hagenfeldt, K.. Intrauterine contraception with the copper T device. 1 Effect on trace elements in the endometrium, cervical mucus and plasma Contraception 6. 37 1972.

6. — Intrauterine contraception with the copper T device. 2. Influence on endometrial acid and alkaline phosphatase, beta-glucuronidase and lactic dehydrogenase activities. Contraception 6. 191 1972.

7 Hagenfeldt, K., Johannisson, E. & Brenner P Intrauterine contraception with the copper T device 3 Effect upon endometrial morphology Contraception 6. 207 1972.

8 Hughes, E. C., Demers, L. M., Csermely T & Jones D B Organ culture of human endometrium. Effect of ovarian steroids. Am J Obst Gynecol 105 707 1969

9 Jeffrey P L., Brown, D. H. & Illingworth Brown, B Studies of lysosomal a-glucosidase II. Kinetics of action of the rat liver enzyme Biochemistry 9 1416, 1970

10 Kanamura S. Ultrastructural localization of glucose phosphatase activity in proximal convoluted tubule cells of rat kidney Histochemie 28 288, 1971.

11 Leskes, A Siekevitz, P & Palade G. E.. Differentiation of endoplasmic reticulum in hepatocytes I. Glucose-6 phosphatase distribution in situ. J Cell Biol 49 264 1971

12. Lopez de la Osa E., Hagenfeldt, K. & Diczfalusy E. Effect of the Cu-T device on the glycogen content of the human endometrium. Contraception 6 44? 1972.

13 Millo, F Lombardi, L. & Gautier A Mise en évidence et ultrastructure du glycogène hépatique Influence des techniques de préparation. J Ultrastruct Res 16 399 1966.

14 Naeslund, G Blastocystotoxic effect of copper in vitro. Contraception 6 ?81 1972.

15 Nilsson, O Electron microscopy of the glandular epithelium in the human uterus. I Follicular phase J Ultrastruct Res 6 413 1962.

16. — Electron microscopy of the glandular epithelium in the human uterus. II Early and late luteal phase J Ultrastruct Res 6 422, 196..

17 — In Nobel Symposium 15 Control of human fertility (ed E. Diczfalusy & U Borell), p. 16 Almqvist & Wiksell, Stockholm, 1971

18 Nilsson, O & Hagenfeldt, K Scanning electron microscopy of human uterine epithelium influenced by the Cu T device. Am J Obstet Gynecol, in press

19 Pfeifer U Zur Stellung des lysosomalen Glykogenabbaues im hepatocellulären Glykogenstoffwechsel. Celluläre Autophagie nach intraperitonealer Injektion hypertoner Glukoselösung Virchow Arch (Zellpathol) 10 108, 197

20 Phillips, M J Doornewaard, G Unakar N J & Steiner J W Glycogen depletion in the newborn rat liver A electron microscopic and electron histochemical study J Ultrastruct Res 18 14.., 1967

1 Robles, F Lopez de la Osa E., Lerner U Johannisson, E., Brenner P., Hagenfeldt, K. & Diczfalusy

R. α-Amylase, glycogen synthetase and phosphorylase in the human endometrium: Influence of the cycle and of the Cu-T device. Contraception *6*: 373, 1972.

Sakuma, S. Glykogengehalt und -verteilung in der Uterusschleimhaut der Frau während des normalen Zyklus im elektronenmikroskopischen Bild. Glycogen content and distribution in the uterine mucous membrane of women during the normal cycle as demonstrated by the electron microscope. Beitr Pathol Anat *140*: 434, 1970.

Sawaragi, I. & Wynn, R. M. Ultrastructural localization of metabolic enzymes during the human endometrial cycle. Obstet Gynecol *34*: 50, 1969.

Schiaffino, S. & Hanzlíková, V. Autophagic degradation of glycogen in skeletal muscles of the newborn rat. J Cell Biol *52*: 41, 1972.

Sengel, A. & Stoebner, P. Ultrastructure de l'endomètre humain normal. II. Les glandes. Z Zellforsch *109*: 260, 1970.

Tatum, H. J. Intrauterine contraception. Am J Obstet Gynecol *112*: 1000, 1972.

Themann, H. Elektronenoptische Untersuchungen über das Glykogen im Zellstoffwechsel. Veröff Morphol Pathol *65*: 32, 1963.

Ullmann, G. & Hammerstein, J. Inhibition of sperm motility in vitro by copper wire. Contraception *6*: 71, 1972.

29. Verhagen, A. & Themann, H. Elektronenmikroskopische Untersuchungen am menschlichen Endometrium unter Einwirkung von Ovulationshemmern mit gleichzeitiger Östrogen- und Gestagenwirkung. Arch Gynaekol *209*: 162, 1970.

30. Wanson, J.-C. & Drochmans, P. Role of the sarcoplasmic reticulum in glycogen metabolism. Binding of phosphorylase, phosphorylase kinase and primer complexes to the sarcovesicles of rabbit skeletal muscle. J Cell Biol *54*: 206, 1972.

31. Wynn, R. M. & Harris, J. A.: Ultrastructural cyclic changes in the human endometrium. I. Normal preovulatory phase. Fertil Steril *18*: 632, 1967.

32. Wynn, R. M. & Woolley, R. S. Ultrastructural cyclic changes in the human endometrium. II. Normal postovulatory phase. Fertil Steril *18*: 721, 1967.

33. Zipper, J. A., Tatum, H. J., Pastene, L. & Medel, M. Metallic copper as an intrauterine contraceptive adjunct to the "T" device. Am J Obstet Gynecol *105*: 1274, 1969.

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VACUUM ASPIRATION IN THE DELIVERY ROOM

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Abstract A vacuum aspirator was used to remove retained fragments of placenta, membranes and hyperplastic decidua after delivery. No post-partum endometritis occurred in the group of 60 patients so treated, whereas five infections were found in control group of 50 patients. The difference is almost significant. Moreover favourable experience has led to the use of the vacuum aspirator in the treatment of atonic uterine haemorrhage. No complications have been observed, and the risk seems to be less than that in conventional curettage, particularly as anaesthesia is unnecessary

The first to report a method of evacuation of the uterine cavity in early pregnancy by vacuum aspiration were Wu-Yan-tai and Wu-Hsien-chen (7) in China in 1958. The method has been used since then particularly in the East European countries (3 8 1) and, in recent years, also elsewhere in the world (4 5)

Nilsson (4) in 1967 was the first to try vacuum aspiration for haemorrhages 3–6 weeks post partum (six cases). It was not until 1971 that a study was published by Magursky & von Friesen (?) (15 cases) in which retained fragments of placenta and membranes were removed by vacuum aspiration immediately post partum without anaesthesia. They employed a special aspiration device with a basket-shaped apex.

Since December 1969 a Russian vacuum aspirator supplied by W/O Medeksport has been used in abortions performed at the Central Hospital of Central Ostrobothnia. W began to use the same aspirator in the autumn of 1970 after deli ery in cases in which the placenta or membranes were ragged or incomplete. We present our own results here and compare the incidence of complications after this procedure with the occurrence of the same symptoms in cases managed conservatively

MATERIAL AND METHOD

Our series consisted of 60 patients of whom 24 were primiparae. The highest parity was XII. The series included two twin pregnancies and two cases with dead fetus. The average duration of delivery was 10 hours 14 min, the second stage of labour lasted 10 min and the third 14 min. The blood loss intra partum was 400 g on average. The average share of the weight of the placenta in the infant's birth weight (IBW) was 19.3%. No anaesthesia was used. The diameter of the vacuum aspirator was 12 mm, and it was thus the biggest abortion vacuum aspirator. The aspiration pressure was 0.6 kg/cm². A sample of each aspirate was sent to the pathologist. No oxytocics were administered after the procedure and no prophylactic antibiotic therapy was given.

The control series consisted of 50 patients whose placenta and membranes were also ragged or incomplete, but in whom evacuation of the uterus was not indicated because the diagnosis was uncertain or because the amount of retained products was thought to be small. The control series did not differ otherwise from the investigation group to any appreciable extent. Eighteen of the 50 controls were primiparae; the highest parity was IX. The average duration of delivery was 11 hours 0 min, that of the second stage 9 min and of the third 10 min. The average blood loss was 250 g. The average share of the weight of the placenta in the infant's birth weight was 18.4%. All these patients received oxytocics by mouth in the [illegible]rd after delivery

RESULTS

Table I shows that in 40 (67%) of the total of 60 cases in the investigation group fragments of placenta or membranes were found at the histological examination of the aspiration material. Twelve patients were given antibiotics because of vacuum extractor delivery incipient mastitis or superficial phlebitis. Not single patient developed clinical endometritis. Ten patients had pyrexia (37.7 C) for at least two days. Ten of the 50 controls (Table II) had pyrexia. The dif

Table I *Symptoms findings treatment and complications of 60 patients treated by post-partum vacuum aspiration*

Finding	Patients	Histological specimen contained fragments of placenta	Histological specimen contained fragments of membranes	Patients given antibiotics	Temperature of > 37 7°C for over two days	Cases with endometritis
Incomplete placenta	9	1	5	2	—	—
Incomplete membranes	16	—	12	4	3	—
Placenta and membranes incomplete	5	—	3	2	1	—
Ragged placenta	16	6	2	1	2	—
Ragged membranes	5	—	4	1	1	—
Placenta and membranes ragged	9	1	6	2	3	—
Total	60	8	32	12	10	0

ference was not statistically significant. There were five cases of proven endometritis in the control group. The difference in the incidence of endometritis between the investigation and control groups was statistically almost significant ($\chi^2 = 4\ 192$, $P < 0\ 05$).

Histological examination disclosed muscle tissue in only two cases of the investigation group. There was not a single case of perforation of the uterus.

The average duration of treatment after delivery was 7.2 days in the investigation group (one patient was under treatment for 19 days for severe symphysiolysis). The average treatment time for persons whose aspirate revealed fragments of placenta or membranes was 7 4 days It was exactly 7 days for the patient with no such remnants in the aspiration material. The average treatment time for the control group was 7 4 days.

CASE REPORTS

Two patients in our series had haemorrhage due to uterine atony Our experience of the use of a vacuum aspirator in the treatment of these cases was favourable, as will appear from the following case reports.

Case 1 The patient was a healthy para III of 31 whose earlier pregnancies and deliveries (1964 and 1967) had been normal. The calculated date of delivery was April 9 1971 The patient was admitted to hospital on April 11 1971 because of uterine contractions. The membranes ruptured spontaneously two hours after the onset of regular contractions. During the stage of dilatation the patient was given 2 × 5 IU of oxytocin (Partocon®) intranasally The duration of the first stage of labour was 2 hours 40 min, of the second stage 10 min and of the third 10 min. The placenta was ragged and displayed non-villous areas, the membranes were intact. After the birth of the child the patient was given 5 IU of oxytocin plus 0.5 mg of methylergometrine maleate (Syntometrine M®) intramuscularly The fundus of the uterus was at the level of the umbilicus after expulsion of the placenta, the uterus was flaccid and the total blood loss was about 600 g. The patient was given an additional 0 2 mg of methylergometrine maleate (Methergin®) intramuscularly The uterus remained flaccid, however and the bleeding continued The uterine cavity was evacuated by aspiration which yielded 700 g of blood and coagula, whereafter the uterus contracted well and the bleeding stopped The patient was given no oxytocic subsequently She made uncomplicated recovery and

Table II *Symptoms and treatment of the 50 patients of the control series*

Finding	Patients	Patients given antibiotics	Temperature of 37 7°C for over two days	Cases with endometritis
Incomplete placenta	4	—	1	—
Incomplete membranes	17	7	5	3
Placenta and membranes incomplete	4	1	1	1
Ragged placenta	12	2	1	1
Ragged membranes	1	—	—	—
Placenta and membranes ragged	12	1		—
Total	50	11	10	5

the lochia was normal. Histological examination revealed congested decidua, there were no fibrin.

Case 2. The patient was a healthy para II aged 23. She had had her first delivery in 1968 and had undergone curettage for reason haemorrhage 2 weeks post partum. The curettage specimen contained placental tissue. According to the patient's history the third stage of labour had been long, but no detailed information was given. The calculated date of delivery was October 7 1970. The patient was admitted to hospital on October 20, 1970, because of postmaturity. Estrogen sensitization therapy was given on October 21 and delivery was induced on the following day by administering 1 070 IU of oxytocin (Partolact®) buccally. The membranes were ruptured when the os of the uterus was open 4–5 cm. The first stage lasted 21 hours 2 min, the second stage 8 min and the third 25 min. The blood loss during and after the third stage of labour totalled 1 000 g. The placenta was ragged, the membranes intact. After the birth of the child, the patient was given 5 IU of oxytocin plus 0.5 mg of methylergometrine maleate intramuscularly and, as the bleeding continued, another 0.2 mg of methylergometrine maleate intramuscularly. As the bleeding still persisted, vacuum aspiration was performed, revealing fragments of placenta on gross examination. The uterus contracted well after this and there was hardly any bleeding. The patient made an uncomplicated recovery. The lochia was normal. Histological examination revealed blood clots and fibrotic placental fragments.

DISCUSSION

Our experience suggests that the use of the vacuum aspirator in the delivery-room is justified, especially as anaesthesia is not necessary. We regard incompleteness or considerable raggedness of the placenta and membranes as the indications. Our results show that when the vacuum aspirator was employed for these indications the incidence of post partum endometritis was lower than in the control group, managed conservatively.

Histological examination of the aspiration material revealed fragments of placenta and membranes in 67 % of the specimens. They revealed, however, a great deal of decidua, so placental and membranous fragments may have existed elsewhere in the aspiration material also in the cases in which none was observed by the pathologist.

The method is useful also for postpartum haemorrhage. It is faster and simpler to perform than routine curettage as no anaesthesia is necessary. The uterine cavity is evacuated more thoroughly than in curettage as aspiration gives better removal of blood clots and blood that have accumulated in the cavity as well as extracting possible fragments of placenta and membranes.

The method appears to be safe. No case of perforation of the uterus occurred in our series. Nor is this complication reported in the vacuum aspiration series of Chalupa consisting of over 14 000 abortions (1). Muscle tissue was encountered at the histological examination in two cases (3 %). The corresponding figure in Nilsson's (4) study was 3.3 % according to Vladov et al. (6) it was 0 % and to Chalupa (1) 1 %. The aspiration pressure we used was 0.6 kg/cm². Magursky & von Friesen (2) used 0.4 kg/cm² in post partum aspirations, whereas in abortion-vacuum aspirations the pressure used was by Nilsson (4) 0.7 kg/cm², by Vladov et al. (6) 0.5–1.0 kg/cm² and by Chalupa (1) it was 0.4 kg/cm². Provided that the aspiration pressure is not raised too much, the risk of synechiae is probably no greater than in conventional curettage. However a repeat study of the same cases is being planned to clarify the incidence of synechiae following vacuum aspiration.

REFERENCES

1. Chalupa, M. Gebrauch des Vakuums zur künstlichen Schwangerschaftsunterbrechung. Zbl Gynäkol 86 1803, 1964.
2. Magursky V & Friesen B von. A special suction-tube for post partum evacuation of the uterus. Acta Obstet Gynecol Scand 50 292, 1971.
3. Malka, E. L & Rosa, L. V. Nov akus ginekol. Riga, 1961.
4. Nilsson, C A. Vacuum-aspiration of uterine contents in legal abortion and allied conditions. Acta Obstet Gynecol Scand 46 501, 1967.
5. Tan, P M. Ratnam, S S & Quek, S P. Vacuum aspiration in the treatment of incomplete abortion. J Obstet Gynaecol Br Commw 76 834 1969.
6. Vladov E. Ivanov I. Angelov A. & Raklovska, I. Schwangerschaftsunterbrechung mit Vakuumaspiration. Gynaecologia (Basel) 159 54 1965.
7. Wu-Yuan-tai & Wu-Hsien-chen. Suction curettage for artificial abort. Preliminary report of 300 cases. Chin J Obstet Gynec 6/5 447 1958. Abstract: Excerpta Med (Amst.) Sect. X, 12 244, 1959.
8. Zubejev A V. Vopr akus ginekol pomos 25 1962.

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Incomplete membranes	16	—	12	4	3	—
Placenta and membranes incomplete	5	—	3	2	1	—
Ragged placenta	16	6	2	1	2	—
Ragged membranes	5	—	4	1	1	—
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Table II *Symptoms and treatment of the 50 patients of the control series*

Finding	Patients	Patients given antibiotics	Temperature of > 37.7°C for over two days	Cases with endometritis
Incomplete placenta	4	—	1	—
Incomplete membranes	17	7	5	3
Placenta and membranes incomplete	4	1	1	1
Ragged placenta	12	2	1	1
Ragged membranes	1	—	—	—
Placenta and membranes ragged	12	1	2	—
Total	50	11	10	5

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ADRENOCORTICAL FUNCTION OF PATIENTS USING ORAL CONTRACEPTIVES

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Abstract The effects of different oral contraceptives on adrenocortical function were investigated by assaying photofluorimetrically the 11-OHCS content of the plasma during synthetic ACTH (Hormactid®) test. The series consisted of 43 women, 27 of whom were taking combined or sequential contraceptives, and 8 low-gestagen pills. The remaining 8 patients acted as controls. The plasma 11-OHCS level in the low-gestagen group was the same as that in the control group both before and during the test. The combined type contraceptive pills containing estrogen and gestagen had an elevating effect on the 11-OHCS level of the plasma. The 11-OHCS level in these groups was significantly higher both before and during the ACTH test than the corresponding level in the control group. The difference is assumed to be due to the increased transcorting content of the plasma and to the increased biological half-life of cortisol induced by estrogen.

Several metabolic changes, e.g. changes in carbohydrate metabolism, have been noted during the use of oral contraceptives (17, 19). The mechanism of the action of oral contraceptives on carbohydrate metabolism is not known exactly but it has been noted that the changes are mainly due to the estrogen component (17, 19). It has been postulated that the rise of total cortisol level (2, 11) and the changes in cortisol kinetics (3) noted after the use of oral contraceptives may be closely related to the disorders of carbohydrate metabolism.

The purpose of the present work was to elucidate the possible changes in adrenocortical function during the use of different oral contraceptives.

MATERIAL

The test was performed on 43 healthy women in the fertile age. Eight of them remained as control group, while 35 used contraceptive pills. The oral contraceptives taken were the estrogen–gestagen combined type, the estrogen–gestagen sequential type and the low-gestagen type (Table I). The control patients were trainee nurses and laboratory nurses. The age distribution was the same in all the groups. Each member of the test group had been taking contraceptive pills for at least two menstrual cycles before the test.

METHOD

Adrenocortical function was measured by the synthetic alpha$^{1-24}$ adrenocorticotrophic hormone (Hormactid®), Ferring Ab, Malmö). The dose of hormone used was 10 µg, administered intramuscularly.

Plasma samples were obtained before and 15, 30, 60 and 120 min after the injection. The test was performed at 10.00–12.00 o'clock a.m. on the 15th–20th day of the menstrual cycle.

The 11-OHCS content of the plasma was assayed photofluorimetrically using a slightly modified method of de Moor et al. (4). The details of the modification have been explained previously (6).

RESULTS

Before the ACTH injection, plasma 11-OHCS level was higher in the group using estrogen–gestagen pills than in the control group (Table II). There was a statistically significant difference between the Delpregnin group and the control group, and an almost significant difference between the groups using Ovanon, Primovlar and E-con on the one hand and the control group on the other. The 11-OHCS level of the Exluton group did not differ from that of the control group.

The results were similar during the ACTH test (Table II). The above-mentioned estrogen–gestagen groups had values significantly higher than

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ADRENOCORTICAL FUNCTION OF PATIENTS USING ORAL CONTRACEPTIVES

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Abstract. The effects of different oral contraceptives on adrenocortical function were investigated by assaying photofluorimetrically the 11-OHCS content of the plasma during synthetic ACTH (Homacthid®) test. The series consisted of 43 women, 27 of whom were taking combined or sequential contraceptives, and 8 low-gestagen pills. The remaining 8 patients acted as controls. The plasma 11-OHCS level in the low-gestagen group was the same as that in the control group both before and during the test. The combined type contraceptive pills containing estrogen and gestagen had an elevating effect on the 11-OHCS level of the plasma. The 11-OHCS level in these groups was significantly higher both before and during the ACTH test than the corresponding level in the control group. The difference is assumed to be due to the increased transcorting content of the plasma and to the increased biological half-life of cortisol induced by estrogen.

Several metabolic changes, e.g. changes in carbohydrate metabolism, have been noted during the use of oral contraceptives (17, 19). The mechanism of the action of oral contraceptives on carbohydrate metabolism is not known exactly, but it has been noted that the changes are mainly due to the estrogen component (17, 19). It has been postulated that the rise of total cortisol level (2, 11) and the changes in cortisol kinetics (3) noted after the use of oral contraceptives may be closely related to the disorders of carbohydrate metabolism.

The purpose of the present work was to elucidate the possible changes in adrenocortical function during the use of different oral contraceptives.

MATERIAL

The test was performed on 43 healthy women in the fertile age. Eight of them remained as control group, while 35 used contraceptive pills. The oral contraceptives taken were the estrogen–gestagen combined type, the estrogen–gestagen sequential type, and the low-gestagen type (Table I). The control patients were trainee nurses and laboratory nurses. The age distribution was the same in all the groups. Each member of the test group had been taking contraceptive pills for at least two menstrual cycles before the test.

METHOD

Adrenocortical function was measured by the synthetic alpha[1–24] adrenocorticotrophic hormone (Homacthid®), Ferring Ab, Malmö). The dose of hormone used was 10 µg, administered intramuscularly.

Plasma samples were obtained before and 15, 30, 60 and 120 min after the injection. The test was performed at 10.00–12.00 o'clock a.m. on the 15th–20th day of the menstrual cycle.

The 11-OHCS content of the plasma was assayed photofluorimetrically using a slightly modified method of de Moor et al. (4). The details of the modification have been explained previously (6).

RESULTS

Before the ACTH injection, plasma 11-OHCS level was higher in the group using estrogen–gestagen pills than in the control group (Table II). There was a statistically significant difference between the Delpregnin group and the control group, and an almost significant difference between the groups using Ovanon, Primovlar and E-con on the one hand and the control group on the other. The 11-OHCS level of the Exluton group did not differ from that of the control group.

The results were similar during the ACTH test (Table II). The above-mentioned estrogen–gestagen groups had values significantly higher than

Table I *The patients and the oral contraceptives*

Preparation	Proprietary name	Oestrogen	Gestagen	No. of patients
Combined type	Delpregnin (Novo)	Mestranol 0.1 mg	Megestrol acetate 5 mg	10
Combined type	E-Con (Lääke Oy)	Ethinylestradiol 0.05 mg	Norethisterone acetate [illegible] mg	5
Combined type	Primovlar (Leiras)	Ethinylestradiol 0.05 mg	Norgestrel 0.5 mg	5
Combined type	Ortho-Novin mite (Ortho)	Mestranol 0.08 mg	Norethisterone 1 mg	1
Combined type	Lyndiol (Organon) (2.5)	Mestranol 0.075 mg	Lynestrenol 2.5 mg	1
Sequential type Low-dose	Ovanon (Organon)	I Mestranol 0.08 mg II Mestranol 0.075 mg	Lynestrenol 2.5 mg	5
Gestagen type	Exluton (Organon)	—	Lynestrenol 0.5 mg	8
Controls		—	—	8
Total number of patients				41

the control group. The adrenal response of the Exluton group was equivalent to that of the control group.

The material was divided into three groups according to the estrogen component (Table III). The *initial level in the groups taking 0.05 mg* of ethinyl-estradiol or 0.1 mg of mestranol was significantly higher and that in the group taking 0.075–0.08 mg of mestranol almost significantly higher than the level in the control group. The results obtained during the ACTH test show that the 11-OHCS level at 30, 60 and 120 min was highly significantly higher in all the three groups than in the control group.

The differences between the initial level and *the highest level recorded during the test,* classified according to the group of preparations and the estrogen component are shown in Table IV. The difference was greatest in the Primovlar group and smallest in the Exluton group, in which the difference was equal to that of the control group.

The percentages of difference between the values of the test groups and those of the control group during the ACTH test (Fig. 1) show that the 11-OHCS level in the Exluton group was the same as that of the control group throughout the experiment. The groups which used estrogen-gestagen preparations had 11-OHCS levels consistently higher by at least 30% than the control level. During the first hour the level was more or less parallel with that of the control group, but between 60 and 120 min the difference increased in all the three estrogen–gestagen

Table II Homactid-test in women taking different oral contraceptives

Comparison with the control material by the students *t*-test 11-OHCS [illegible] µmol/l (35.7 µmol/l µg 100 ml)

Preparation	No.		0	15	30	60	120 min
Controls	8	M	0.51	0.72	0.81	0.83	0.57
		S.D	0.21	0.09	0.13	0.15	0.15
Delpregnin	10	M	0.89	1.22	1.41	1.48	1.39
		S.D	0.29	0.30	0.34	0.22	0.29
E-Con	5	M	0.85	0.23	1.51	1.41	0.97
		S.D	0.28	0.23	0.42	0.43	0.30
Primovlar	5	M	0.80	1.21	1.54	1.43	1.23
		S.D	0.18	0.42	0.29	[illegible]	0.16
Ovanon	5	M	0.81	0.95	1.14	1.79	1.25
		S.D	0.21	0.22	0.10	0.13	0.22
Exluton	8	M	0.47	0.66	0.76	0.68	0.55
		S.D	0.13	0.16	0.16	0.10	0.18

– not significant nearly significant $p < 0.05$ significant p 0.01 highly significant p 0.001

Table III. *The results of the Homoctid-test in different groups according to the estrogen component*

Comparison with the control material by the students-test. 11-OHCS value µmol/l

Estrogen	No		0	15	30	60	120 min
Controls	8	*M*	0.51	0.72	0.81	0.83	0.57
		S.D.	0.21	0.09	0.13	0.15	0.15
Ethynylestradiol 0.05 mg	10	*M*	0.83	1.30	1.42	1.46	1.13
		S.D.	0.23	0.31	0.36	0.35	0.26
Mestranol 0.1 mg	10	*M*	0.99	1.22	1.41	1.48	1.39
		S.D.	0.29	0.30	0.34	0.22	0.29
Mestranol 0.75–0.80 mg	7	*M*	0.78	0.93	1.10	1.27	1.13
		S.D.	0.18	0.19	0.11	0.13	0.27

not significant   nearly significant $p < 0.05$ significant $p < 0.01$ highly significant $p < 0.001$

groups. This increase was most noticeable in the mestranol groups.

DISCUSSION

The test of adrenocortical function with synthetic adrenocorticotrophic hormone has previously been established as applicable for clinical diagnostic use (6, 18, 21, 22). The results obtained in this way are similar to those obtained with natural ACTH hormone (5).

The fluorimetric method of assaying 11-OHCS now employed has been established as reliable and applicable to clinical use (14, 16). The accuracy of the method of de Moor et al. is given by the standard deviation of 0.2–0.4 µg/100 ml of plasma (14). Most of the 11-OHCS thus assayed consists of cortisol, another significant component being corticosterone (10).

The present work showed that the plasma 11-OHCS level before the ACTH test was the same in the group receiving 0.5 mg lynestrenol as in the control group. The groups of patients using combined or sequential type contraceptives had higher

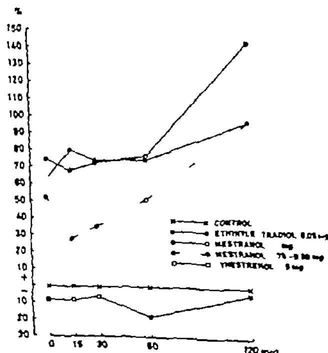


Fig. 1. The percentages of difference from the control group during the ACTH test in the groups classified according to the estrogen component of the contraceptive and in the low-estrogen (Exluton) group.

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Sequential type Low-dose	Ovanon (Organon)	I Mestranol 0.08 mg II Mestranol 0.075 mg	Lynestrenol 2.5 mg	5
Gestagen type	Exluton (Organon)	—	Lynestrenol 0.5 mg	8
Controls		—	—	8
Total number of patients				43

the control group. The adrenal response of the Exluton group was equivalent to that of the control group

The material was divided into three groups according to the estrogen component (Table III) The initial level in the groups taking 0.05 mg of ethinyl-estradiol or 0.1 mg of mestranol was significantly higher and that in the group taking 0.075–0.08 mg of mestranol almost significantly higher than the level in the control group. The results obtained during the ACTH test show that the 11-OHCS level at 30 60 and 120 min was highly significantly higher in all the three groups than in the control group.

The differences between the initial level and the highest level recorded during the test, classified according to the group of preparations and the estrogen component, are shown in Table IV The difference was greatest in the Primovlar group and smallest in the Exluton group, in which the difference was equal to that of the control group.

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		S.D	0.21	0.09	0.13	0.15	0.15
Delpregnin	10	*M*	0.89	1.22	1.41	1.48	1.99
		S.D	0.29	0.30	0.34	0.22	0.79
E-Con	5	*M*	0.85	0.23	1.51	1.41	0.97
		S.D	0.28	0.23	0.4	0.43	0.30
Primovlar	5	*M*	0.80	1.21	1.54	1.43	1.[illegible]3
		S.D	0.18	0.[illegible]	0.29	0.[illegible]1	0.16
Ovanon	5	*M*	0.81	0.95	1.14	1.29	1.[illegible]5
		S.D	0.21	0.22	0.10	0.13	0.[illegible]
Exluton	8	*M*	0.47 –	0.66 –	0.76 –	0.68 –	0.55
		S.D	0.13	0.16	0.26	0.10	0.18

– not significant nearly significant $p < 0.05$ significant $p < 0.01$ *highly significant* p 0.001

4 De Moor, P Steeno, O Raskin, M. & Hendrikx, A Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. Acta Endocrinol (Kbh) 33, 297, 1960.

5 Holst Larsen, K. & Ulrich, J Comparison between β 1-24 corticotrophin, DW 75 (Sandoz) and natural corticotrophin in normal adults pretreated with dexamethasone. Acta Endocr (Kbh) 66, 627 1971

6 Kauppila, A Hartikainen A-L. & Rehalla, M. Adrenal response to synthetic adrenocorticotrophic hormone during pregnancy and after delivery with special reference to pre-eclamptic and hypertensive pregnancies. Scand J Clin Lab Invest 31, 179 1973.

7. Keane, P M Pearson, J & Walker W H. C. Binding characteristics of transcortin in human plasma in normal individuals, pregnancy and liver disease. J Endocrinol 43 571, 1969

8 Larsson-Cohn, U. Contraceptive treatment with low doses of gestagen. Acta Universitatis Upsaliensis 80, Soderstrom & Plan, Uppsala, 1970.

9 Laurell, C.-B Kullander S. & Thorell, J Effect of administration of combined estrogen-progestin contraceptive and the level of individual plasma proteins. Scand J Clin Lab Invest 21 337 1968

10 Martin, M. M & Martin, A L. A Simultaneous fluorometric determination of cortisol and corticosterone in human plasma. J Clin Endocrinol Metab 28 137 1968.

11 Metcalf M G & Beaven, D. W Plasma-corticosteroid levels in women receiving oral contraceptive tablets Lancet 2 1095 1963

12 Mills, I H Schedl H. P Chen, P S. & Bartter F C The effect of estrogen administration on the metabolism and protein binding of hydrocortisone J Clin Endocrinol Metab 20 515 1960.

13 Musa, B U Ulyses, S S & Doe, R. P Elevation of certain plasma proteins in man following estrogen administration A dose-response relationship J Clin Endocrinol Metab 25 1163, 1965.

14 Nielsen, E. & Asfeldt, V H. Studies on the specificity of fluorimetric determination of plasma corticosteroids ad modum De Moor & Steeno. Scand J Clin Lab Invest 20 185 1967

15 Sandberg, A. A. & Slaunwhite W R., Jr T anscortin. corticosteroid-binding protein of plasma. II Levels in various conditions and the effects of estrogens. J Clin Invest 38 1290 1959

16 Sawin, C. T Measurement of plasma cortisol in the diagnosis of Cushing' syndrome Ann Intern Med 68 624 1968

17 Spellacy W N A review of carbohydrate metabolism and the oral contraceptives. Am J Obstet Gynecol 104 448 1969

18 Still, L., Rehalla M. & Jarvinen, P Adrenocorticotrophin-test applied on women taking oral contraceptives. Acta Endocrinol (Kbh) 67 Suppl. 155 167 1971

19 Sunderman, F W Jr Drug interference in clinical biochemistry CRC Crit Rev Clin Lab Sci 1 427 1970.

20. Tait, J F & Burstein, S In-vivo studies of steroids dynamic in man. In The Hormones, ol. V pp. 441–557 (ed C Pincus, K. V Thimann and F B. Astwood) Academic Press, London, 1964

21. Weber, C. Über den Synacthen-Kurztest. Praxis 58, 861, 1969

22 Woodings, D F & Young, P A rapid test of adrenocortical function using Humactid (synthetic α_1^{1-24} ACTH). Acta Endocrinol (Kbh) 67 470, 1971

23 Wynn, V & Doar J W H. Some effects of oral contraceptives on carbohydrate metabolism. Lancet 2 715, 1966.

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Table IV *Greatest rise of 11 OHCS level (μmol/l) from the initial value in the Homactid tests of the different groups*

Group	No	Rise
Controls	8	0.32
Delpregnin	10	0.59
Ovanon	5	0.48
E-Con	5	0.56
Primovlar	5	0 74
Exluton	8	0.29
Ethinylestradiol 0 05 mg	10	0.63
Mestranol 0.1 mg	10	0.59
Mestranol 0 075–0 080 mg	7	0.49

11-OHCS levels than the control group Estrogen is known to effect a rise in the cortisol-binding protein transcortin (9 12, 13 15). The degree of elevation of the plasma concentration of transcortin has been shown to be related, within limits, to the dose of estrogen (13) The elevation of inactive Cortisol-bound protein is mainly responsible for the increase of total cortisol during preg nancy (1 7) Burke (2) noted that the total cor tisol level of the plasma was higher than normal in all users of oral contraceptives. Unbound cor tisol levels were in the normal range, but the median value was significantly increased. It is apparent that the elevation of plasma cortisol level also noted in this work is a secondary consequence of the estrogen stimulated increase of transcortin.

Adrenocortical response to synthetic ACTH was similar in the control group and the group using Exluton. The groups using combined and sequential type contraceptives showed a more intensive response of longer duration (Fig. 1 Table IV). The difference can assumably be due to two factors. Firstly the half life of cortisol has been found to increase under the effect of estrogen (20). Secondly large amounts of the transcortin due to the effect of estrogen may be present in circulation and bind the cortisol secreted into the blood after ACTH stimulation. The excess of transcortin in the control patients and users of low-gestagen pills is small. Transcortin is therefore quickly used and the part of cortisol remaining free is eliminated rapidly Fig. 1 shows that the difference from the control group re mains more or less constant during the first hour while the values obtained at 120 min indicate a considerable increase in the groups taking co[illegible] traceptive pills with estrogen. Comparison of th different mestranol groups shows the effect of th different doses of estrogen. It seems that as th amount of estrogen increases, the 11-OHCS lev of the plasma rises and the adrenocortical r sponse becomes stronger In the group tak[illegible] 0.07–0 08 mg of mestranol both the initial le was lower and the increase during the ACT test was smaller than in the group taking 1 0 n of mestranol.

It seems that lynestrenol does not, at least wh administered in small doses, affect the plas 11-OHCS level or adrenocortical function. T effect of the gestagen components of the oth preparations could not be assessed in the present study because the effect of estrogen on the 11-OHCS level was so prominent. According to the literature the effect of gestagen on carbohydrate metabolism is very small (8, 17).

Is there any clinical significance in the rise of the cortisol (mainly bound cortisol) level brought about by oral contraceptives? The question is still without a final answer Fell & Weinges (3) came to the following conclusion in their work that it is cortisol which appears to produce the metabolic picture seen following estrogen-gestagen oral contraception. This is present in greater amounts bound to C.B G (Cortisol Binding Globulin) which is known to increase following estrogen therapy The consequent changes in metabolism seem irreconcilable with 'bound' cor tisol since it is biologically inactive, but perhaps this is a paradox. Though inactive it is protected against metabolism and can thus, as it dissociates, maintain a more sustained level of cortisol at a tissue level than the free This could also ex plain why the metabolic change noted in association with estrogen–gestagen oral contraceptives is similar to that seen in corticosteroid-induced diabetes (23)

REFERENCES

1 Aarskog, D Cortisol in the newborn infant Acta Paediat Scand, Suppl. *158* 1965
2. Burke, C W Biologically acth cortisol in plasma of oestrogen-treated and normal subjects. B Med J 798, 1969
3 Fell, P J & Weinges, K. F The early hanges in metabolism in healthy women receiving oral contraception. I print.

4 De Moore, P Steeno, O Raskin, M. & Hendrikx, A. Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. Acta Endocrinol (Kbh) 33 297 1960.

5. Hels q Larsen, K. & Ulrich, L. Comparison between β 1-24 corticotrophin, DW 75 (Sandoz) and natural corticotrophin in normal adults pretreated with dexamethasone Acta Endocr (Kbh) 66 627 1971.

6 Kauppila, A Hartikainen, A. L. & Rehdla, M. Adrenal response to synthetic adrenocorticotrophic hormone during pregnancy and after delivery with special reference to pre-eclamptic and hypertensive pregnancies Scand J Clin Lab Invest 31 179 1973

7 Keane, P M Pearson, J & Walker W H C. Binding characteristics of transcortin in human plasma in normal individuals, pregnancy and liver disease J Endocrinol 43 571 1969

8. Larsson-Cohn, U Contraceptive treatment with low doses of gestagen Acta Universitatis Upsaliensis 80, Soderström & Finn, Uppsala, 1970

9 Laurell, C. B Kullander S & Thorell, J Effect of administration of combined estrogen-progestin contraceptive and the level of individual plasma proteins Scand J Clin Lab Invest 21 337 1968

10 Martin, M M. & Martin, A L. A Simultaneous fluorometric determination of cortisol and corticosterone in human plasma J Clin Endocrinol Metab 28 137 1968.

11. Metcalf, M G & Beaven, D W Plasma-corticosteroid levels in women receiving oral contraceptive tablets. Lancet 2 1095 1963.

12 Mills, I. H Schedl, H. P Chen, P S. & Bartter F C The effect of estrogen administration on the metabolism and protein binding of hydrocortisone. J Clin Endocrinol Metab 20 515 1960

13 Musa, B U Ulyssa, S S & Doe, R. P Elevation of certain plasma proteins in man following estrogen administration: A dose-response relationship. J Clin Endocrinol Metab 25 1163, 1965

14 Nielsen, E & Asfeldt, V H Studies on the specificity of fluorimetric determination of plasma corticosteroids ad modum De Moor & Steeno. Scand J Clin Lab Invest 20 185 1967

15 Sandberg, A. A. & Slaunwhite W R., Jr Transcortin, corticosteroid-binding protein of plasma II. Levels in various conditions and the effects of estrogens. J Clin Invest 38 1290 1959

16 Sevin, C. T Measurement of plasma cortisol in the diagnosis of Cushing's syndrome. Ann Intern Med 68 624 1968

17 Spellacy W N A review of carbohydrate metabolism and the oral contraceptives. Am J Obstet Gynecol 104 448, 1969

18 Sulf, L., Rehdla, M. & Jarvinen, P Adrenocorticotrophin-test applied on women taking oral contraceptives. Acta Endocrinol (Kbh) 67 Suppl. 155 167 1971.

19 Sunderman, F W Jr Drug interference in clinical biochemistry CRC Crit Rev Clin Lab Sci 1 427 1970.

20. Tait, J F & Burstein, S In-vivo studies of steroids dynamic in man *I* The Hormones, vol. V pp 441 557 (ed C. Pincus, K. V Thimann and E. B Astwood) Academic Press, London, 1964

21. Weber, C., Über den Synacthen-Kurztest. Praxis 58 841 1969

22 Woodings, D F & Young, P A rapid test of adrenocortical function using Hormactid (synthetic α_{1-18} ACTH). Acta Endocrinol (Kbh) 67 470, 1971.

23 Wynn, V & Doar J W H. Some effects of oral contraceptives on carbohydrate metabolism. Lancet 2 715 1966.

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OVARIAN MORPHOLOGY AND PITUITARY GONADOTROPHINS IN SERUM DURING AND AFTER LONG-TERM TREATMENT WITH ORAL CONTRACEPTIVES

Jørgen Starup and Jakob Visfeldt

From the Department of Gynaecology and Obstetrics (Heads: Erling Østergaard and Paul Erik Lebech), and the Department of Pathology (Heads: Simon Fischer and Jakob Visfeldt), Frederiksberg Hospital, Copenhagen, Denmark

Abstract. The aim of the present investigation as to di-rectly correlate the development of ovarian follicles with the levels of FSH and LH in serum during long-term treatment with oral contraceptives in order to discover to hat extent follicular maturation beyond the stage of early primary follicles depends upon stimulation with pituitary gonadotropins

Seventeen women aged 20 to 44 ere treated cyclically for 6 to 100 months ith different oral contraceptives of the combination type Group I (10 women) ere ex-amined during treatment, hile group II (7 omen) were examined ithin the first 7 eeks after cessation of treatment Laparotomy was performed in all cases, and FSH and LH in serum were determined radioimmuno-logically shortly before the operation The ovaries were inspected macroscopically and large edge excisions were submitted to histological examination.

During treatment the values of serum FSH ere low but ithin the normal range in all cases The alues of serum LH are very low in the normal range or even below the lower limit The number of primordial follicles seemed to be mainly dependent on the age of the individual women, hereas no correlation with the duration of treatment was found All women except one ealed primary follicles in different stages of develop-ment Mor surprising as the fact that 5 out of the 10 women showed antral and/or Graafian follicles in spite of the low levels of FSH and LH No fresh corpora lutea ere found T women showed slight thicken-ing of tunica albuginea, but in no case as perifol-licular fibrosis found

Within the first 7 eeks after treatment 6 out of the 7 women revealed antral and or Graafian follicles, and 2 of them fresh corpus luteum In addition, all val-ues of FSH and LH in serum were ithin the normal range and significantly higher than during treatment

In spite of the fact that oral contraceptives of the combination type ha e now been in clinical use since 1956, information on the ffects of these agents on ovarian morphology in women is rather sparse. The literature has been critically reviewed, and we found only 10 studies with an adequate description of the development of ovarian follicles during treatment (8 9 10 14 15 18, 19 21 24 25). The observations seem to be contradictory but this might be explained by the fact that many different hormonal prepara-tions have been used and for varying periods of time. Furthermore, the majority of series con-sists of only a small number of women, which makes it very difficult to draw any conclusions. In the largest series so far it was found by Maqueo et al (10) that 27 out of the 60 women investigated showed primary follicles in different stages, while 5 women revealed antral follicles, and only 2 women showed Graafian follicles. None of these 60 women revealed any fresh cor pora lutea. The latter observation is in agreement with those of many other investigators (4 6, 10 14 15 18, 19 21 24 25 26). Only Ludwig (8) and Mall-Haefeli et al. (9) have in a few cases observed apparently fresh corpora lutea dur ing the first 2 months of treatment, but the ac tivity of these corpora lutea was not normal, since the urinary excretion of pregnanediol was low In addition to this more or less pronounced inhibition of the follicular development, several authors have described thickening of tunica albuginea (2 10, 18 21) and perifollicular fi-brosis (4 10 14 18 25) during long-term treat ment with oral contraceptives.

It is now generally agreed that inhibition of pituitary gonadotropin secretion is the most im-

Table I *Treatment and serum gonadotropins in group I*

Pat. no	Age (yrs)	Preparation	Duration of therapy (months)	FSH (mIU/ml)	LH (mIU/ml)
1	40	Gestovex	7	9	4
		Lyndiol	5		
2	33	Eugynon	6	10	4
3	26	Lyndiol	72	5	< 1
4	20	Ovulen	18	13	6
5	38	Lyndiol	48	6	2
		Ovulen	52		
6	40	Neodel-pregnin	9	4	8
7	23	Lyndiol	6	—	—
8	39	Lyndiol	6	—	—
9	29	Lyndiol	14	4	< 1
10	22	Nonovul	11	8	5

portant mode of action of the oral contraceptives. This was primarily shown by the observation that the ovarian response to administration of exogenous gonadotropins was not inhibited in women who were treated simultaneously with oral contraceptives (e.g. 5 15 16, 22). Recently however the specific and sensitive radioimmunoassays for determination of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in both serum and urine have been more direct tools in the solution of these problems. Using radioimmunoassays Goldzieher et al. (3) found that long-term treatment with oral contraceptives did not only cause an abolition of the midcycle surge of both FSH and LH but did also during the remaining part of the cycle, suppress plasma FSH to about 70% of the control values and plasma LH to 20–30% of the control values. The usual difference between the "follicular and luteal" phase levels of FSH and LH was, however preserved during treatment. The values for both FSH and LH in plasma returned to normal levels within the first 3 months after cessation of treatment. These findings fully explain the observations by Lopez Llera et al. (7) and Weber (23) who found evidence of normal follicular maturation and ovulation in the majority of ovarian biopsies obtained during the first 3 months after the treatment had been discontinued even in women treated for several years.

The purpose of the present investigation has been directly to correlate the ovarian morphology during and after long-term treatment with oral contraceptives with the concentrations of FSH and LH in serum. An investigation of this rel tion seems important since it is still not certa to what extent follicular growth and maturatic beyond the stage of early primary follicles de pends upon stimulation with pituitary gonad tropins. Furthermore, to our knowledge a simil study has not yet been carried out.

MATERIAL AND METHODS

Seventeen women aged 20 to 44 (in average 33) we treated cyclically for a period of 6 to 100 months (average 34 months) with different oral contraceptives the combination type The series was divided in grou Group I consisted of 10 women, who were exam during treatment, while group II consisted of the r maining 7 women, who ere all examined within t first 7 weeks after cessation of treatment. Tables I a III show age hormonal treatment and duration of the apy for the individual woman in groups I and II, r spectively It appears that all women received oral co traceptives of the combination type and that 10 one were treated with only one preparation, while 7 wom received 2 different preparations during the treatmer period. Furthermore T ble III shows that out of th 7 women in group II one was examined 14 days aft treatment, four 21 days after treatment, one 4 da after treatment and the last one 49 days after treatm

In 15 out of the 17 women the concentration o FSH and LH in serum was determined radioimmunolo cally by the double antibody method described b Midgley (11 12) and Midgley et al. (13), only slight modified according t incubation time and volumes. Th pituitary preparation LER 907 from NIH as used a standard and the results e pressed in terms of th Second International Reference Preparation of Hum Menopausal Gonadotropin (2nd IRP HMG) For LH th means that 1 mg LER 907 is equivalent to 700 IU o the 2nd IRP HMG and for FSH that 1 mg LER 90 is equivalent to 33 IU of the 2nd IRP HMG All valu of FSH and LH gi en are means of double determina tions, and are expressed in mIU of the 2nd IRP-HMG ml serum. In the ovulatory cycle the normal range o FSH is 4 to 53 mIU/ml serum, and the normal rang of LH is 3 to 154 mIU/ml serum

Lapa otomy was performed in all 17 women. The ovar les were inspected macroscopic lly and large wedge ex cisions were submitted to histological examination. Th specimens were fixed in formalin, blocked sectioned nd stained both ith hematoxylin-eosin and with th van Gieson m thod in order to differentiate connec ti tissue from the ov ian stroma

The follicles were classified a primordial follicles, pri mary follicles, antral follicles, Graafian follicles and atretic follicles according to the following crit ia. A primordial follicle consists of a oocyte surrounded by a single layer of flattened spindle-shaped ells and i separated f om the surrounding ovarian stroma by a definite basement membrane The term primary follicle pplies t all follicles from the moment hen the flat

Table II. *Ovarian morphology in group I*

ent	Primordial foll.	Primary foll.	Antral foll.	Graafian foll.	Fresh corp. lut.	Corp. albicantia	Atretic foll.	Follicular cysts	Thickening of tunica albuginea	Perifollicular fibrosis
1		+	–	–	–	+	+	+	–	–
2		+	+	–	–	+	+	+	(+)	–
3				+	–	–	+	+	–	–
4			+	+	–	+		+	–	–
5			–	–	–	–	+	–	(+)	–
6			–	+	–	+	+	+	–	–
7			–	–	–	+	–	–	–	–
8				–	–	–	+	–	–	–
9			–	–	–	–		+	–	–
10			–	+	–	–		+	–	–

knife-shaped cells inside the basement membrane become cuboidal and until the moment when the first small fluid filled cleft-like spaces appear among the multilayered granulosa cells. When these spaces become confluent, the follicle has reached the stage of an antral follicle, and finally the follicle enlarges and matures to Graafian follicle. Atresia may occur at any stage of the follicular development, and all these degenerating follicles are called atretic follicles. The number of primordial follicles was roughly estimated and was recorded as few ( ), several ( ) or many (+++), whereas the more developed follicles are only recorded as present or not present. In addition, all ovarian biopsies are examined for fresh corpora lutea, corpora albicantia, follicular cysts, thickening of tunica albuginea and perifollicular fibrosis.

RESULTS

Table I shows that the values of serum FSH during treatment varied *from 4 to 13 mIU/ml*, i.e. that the level of FSH was low but within the normal range in all women investigated. It also appears from Table I that the values of serum LH in group I varied from <1 to 8 mIU/ml, i.e. that the level of LH during treatment was very low in the normal range or even below the lower limit. Because of the small size of the series it is not possible to determine, whether there is a correlation between the levels of FSH and LH and the length of the treatment period.

Table II shows the ovarian morphology during treatment. The number of primordial follicles varied considerably but seemed to be mainly dependent on the age of the individual woman, whereas no correlation with the duration of therapy could be demonstrated. During treatment all women except one (no. 8) showed primary fol-



Fig. 1. Ovarian biopsy from 33-year-old woman (no. 2) treated with Engynon for 6 months. Several primordial follicles and one primary follicle are seen in the normal ovarian stroma (H E. 120).

Table I *Treatment and serum gonadotropins in group I*

Pat no.	Age (yrs)	Preparation	Duration of therapy (months)	FSH (mIU/ml)	LH (mIU/ml)
1	40	Gestovex	7	9	4
		Lyndiol	5		
2	33	Eugynon	6	10	4
3	26	Lyndiol	72	5	<1
4	20	Ovulen	18	13	6
5	38	Lyndiol	48	6	2
		Ovulen	52		
6	40	Neodel-pregnin	9	4	8
7	23	Lyndiol	6	—	—
8	39	Lyndiol	6	—	—
9	29	Lyndiol	14	4	<1
10	22	Noneovul	11	8	5

portant mode of action of the oral contraceptives. This was primarily shown by the observation that the ovarian response to administration of exogenous gonadotropins was not inhibited in women, who were treated simultaneously with oral contraceptives (e.g. 5 15 16 22). Recently however the specific and sensitive radioimmunoassays for determination of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in both serum and urine have been more direct tools in the solution of these problems. Using radioimmunoassays Goldzieher et al. (3) found that long term treatment with oral contraceptives did not only cause an abolition of the midcycle surge of both FSH and LH, but did also during the remaining part of the cycle, suppress plasma FSH to about 70% of the control values and plasma LH to 20–30% of the control values. The usual difference between the "follicular" and luteal" phase levels of FSH and LH was, however preserved during treatment. The values for both FSH and LH in plasma returned to normal levels within the first 3 months after cessation of treatment. These findings fully explain the observations by Lopez Llera et al (7) and Weber (23) who found evidence of normal follicular maturation and ovulation in the majority of ovarian biopsies obtained during the first 3 months after the treatment had been discontinued, even in women treated for several years.

The purpose of the present investigation has been directly to correlate the ovarian morphology during and after long-term treatment with oral contraceptives with the concentrations of FSH and LH in serum. An investigation of this relation seems important, since it is still not certain to what extent follicular growth and maturation beyond the stage of early primary follicles depends upon stimulation with pituitary gonadotropins. Furthermore, to our knowledge a similar study has not yet been carried out.

MATERIAL AND METHODS

Seventeen women aged 20 to 44 (in average 33) were treated cyclically for a period of 6 to 100 months (in average 34 months) with different oral contraceptives of the combination type. The series was divided in groups. Group I consisted of 10 women who were examined during treatment while group II consisted of the remaining 7 women, who were all examined within the first 7 weeks after cessation of treatment. Tables I and III show age, hormonal treatment and duration of therapy for the individual woman in groups I and II, respectively. It appears that all women received oral contraceptives of the combination type and that 10 women were treated with only one preparation, while 7 women received 2 different preparations during the treatment period. Furthermore, Table III shows that out of the 7 women in group II one was examined 14 days after treatment, four 21 days after treatment, one 4? days after treatment and the last one 49 days after treatment.

In 15 out of the 17 women the concentration of FSH and LH in serum was determined radioimmunologically by the double antibody method described by Midgley (11 12) and Midgley et al. (13), only slightly modified according to incubation time and volumes. The pituitary preparation LER 907 from NIH was used as standard, and the results expressed in terms of the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG). For LH this means that 1 mg LER 907 is equivalent to 700 IU of the 2nd IRP HMG and for FSH that 1 mg LER 907 is equivalent to 33 IU of the 2nd IRP HMG. All values of FSH and LH given are means of double determinations, and are expressed in mIU of the 2nd IRP-HMG per ml serum. In the ovulatory cycle the normal range of FSH is 4 to 53 mIU/ml serum, and the normal range of LH is 3 to 154 mIU/ml serum.

Laparotomy was performed in all 17 women. The ovaries were inspected macroscopically and large edge excisions were submitted to histological examination. The specimens were fixed in formalin, blocked, sectioned and stained both with hematoxylin-eosin and with van Gieson method in order to differentiate connective tissue from the ovarian stroma.

The follicles were classified as primordial follicles, primary follicles, antral follicles, Graafian follicles and atretic follicles according to the following criteria. A primordial follicle consists of an oocyte surrounded by a single layer of flattened spindle-shaped cells and is separated from the surrounding ovarian stroma by a definite basement membrane. The term primary follicle applies to all follicles from the moment when the

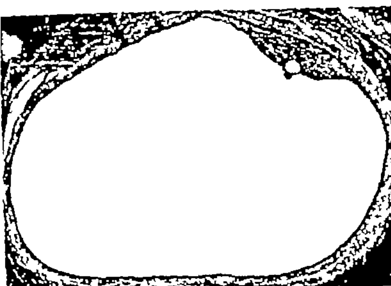


Fig. 4. Ovarian biopsy from 26-year-old oman (no. 5) treated ith Lyndiol for 7? months showing one Graafian follicle in the normal ovarian stroma (H.E. 28).

varied from 4 to 39 mIU/ml. Three out of the 7 women had still rather low levels of LH, but the values were in all cases within the normal range, and for the whole group significantly higher than during treatment (group I). The series is too small to determine, whether there is a correlation especially between the level of LH and the number of days after treatment was discontinued.

Table IV shows the ovarian morphology after treatment. Again, the number of primordial follicles seemed to be mainly dependent on the age of the individual woman. In this group all women showed primary follicles in different stages. Furthermore, it appears that 3 women revealed antral follicles, and 5 women showed Graafian follicles, i.e. that 6 out of the 7 women showed advanced follicular development within the first 7 weeks after cessation of treatment. The next column in Table IV shows that 2 out of the 7 women (nos. 12 and 13) revealed a fresh corpus luteum, respectively 49 and 42 days after the therapy had been stopped. The majority of women showed atretic follicles, and small follicular cysts were also in this group a common finding. Thickening of tunica albuginea and perifollicular fibrosis were not found in any case.

DISCUSSION

The present investigation has confirmed that there are no consistent changes in the ovarian morphology in women during long-term treat

Table III. *Treatment and serum gonadotrophins in group II*

Patient no.	Age (years)	Preparation	Duration of therapy (months)	Days after cessation of therapy	FSH (mIU/ml)	LH (mIU/ml)
11	23	Anovlar	8	21	11	15
		Gestranol	15			
12	44	Gynovlar	60	49	15	15
13	42	Gestovex	43	42	18	39
		Neodelprogestin	36			
14	40	Eugynon	12	14	12	8
		Neodelprogestin	16			
15	38	Cyclored	43	21	11	4
		Duoluton	2			
16	29	Lyndiol	64	21	13	9
		Gestranol	3			
17	37	Gestovex	6	21	17	12

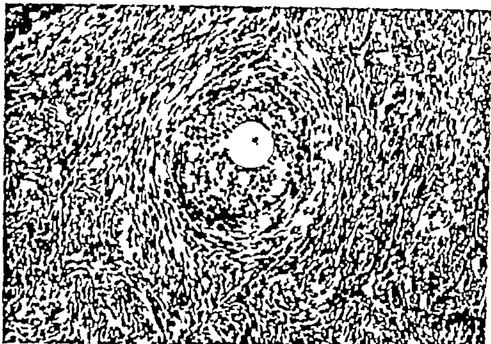


Fig. 2. Ovarian biopsy from a 20-year-old woman (no 4) treated with Ovulen for 11 months showing one primary follicle in the normal ovarian stroma (H.E. × 120).

licles in different stages of development (Figs. 1 and 2). As it appears from the next 2 columns in Table II 3 women revealed antral follicles (Fig. 3), and 4 women showed typical Graafian follicles (Fig. 4), i.e. that 5 out of the 10 women showed greatly developed follicles during long term treatment in spite of the low levels of both FSH and LH in serum. However no fresh corpora lutea were found, but 5 women revealed corpora albicantia. Atretic follicles were found in the majority of cases, and roughly estimated the number of these follicles was normal. Small follicular cysts were also a frequent finding. Finally 2 women showed a slight thickening of tunica albuginea, but in no case was perifollicular fibrosis found.

Table III shows that the values of serum FSH in group II varied from 11 to 18 mIU/ml, i.e. that the level of FSH was normal in all women, and for the whole group significantly higher than during treatment (group I). In addition, it appears that the values of serum LH in group II

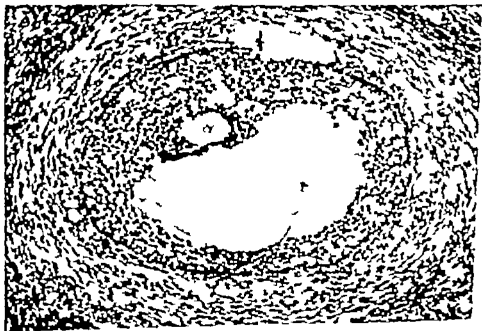


Fig. 3. Ovarian biopsy from a 33-year-old woman (no 2) treated with Eugynon for 6 months showing one antral follicle in the normal ovarian stroma (H.E. × 120).

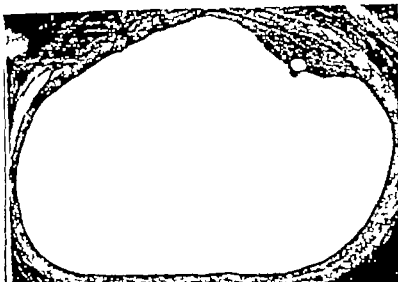


Fig. 4. Ovarian biopsy from 26-year-old woman (no. 3) treated with Lyndiol for 72 months showing one Graafian follicle in the normal ovarian stroma (H.E. ×28).

aried from 4 to 39 mIU/ml. Three out of the 7 women had still rather low levels of LH, but the values were in all cases within the normal range, and for the whole group significantly higher than during treatment (group I). The series is too small to determine, whether there is a correlation especially between the level of LH and the number of days after treatment was discontinued.

Table IV shows the ovarian morphology after treatment. Again, the number of primordial follicles seemed to be mainly dependent on the age of the individual woman. In this group all women showed primary follicles in different stages. Furthermore, it appears that 3 women revealed antral follicles, and 5 women showed Graafian follicles, i.e. that 6 out of the 7 women showed advanced follicular development within the first 7 weeks after cessation of treatment. The next column in Table IV shows that 2 out of the 7 women (nos. 12 and 13) revealed a fresh corpus luteum, respectively 49 and 42 days after the therapy had been stopped. The majority of women showed atretic follicles, and small follicular cysts were also in this group a common finding. Thickening of tunica albuginea and perifollicular fibrosis were not found in any case.

DISCUSSION

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Patient no.	Age (years)	Preparation	Duration of therapy (months)	Days after cessation of therapy	FSH (mIU/ml)	LH (mIU/ml)
11	23	Anovlar	8	21	11	15
		Gestrol	15			
12	44	Gynovlar	60	49	15	15
13	42	Gestovex	48	42	18	39
14	40	Neodelpregnin	34			
		Eugynon	12	14	12	8
15	38	Neodelpregnin	16			
		Cyclovral	48	21	11	4
16	29	Duoluton	2			
		Lyndiol	66	21	13	9
17	37	Gestrol	3			
		Gestovex	6	21	17	12

Table IV *Ovarian morphology in group II*

Patient no	Primordial foll.	Primary foll.	Antral foll.	Graafian foll.	Fresh corp. lut.	Corp albica tia	Atretic foll.	Follicular cysts	Thickening of tunica albuginea	Perifollicular fibrosis
11	+ +	+	–	+	–	–	–	–	–	–
12	+	+	+	–	+	–	–	–	–	
13	+	+	–	+	+	–	+	+	–	–
14	+	+	–	–	–	+	+	–	–	–
15	+	+	–	+	–	–	+	+	–	
16	+ +	+	+	+	–	–	+	–	–	–
17	+ +	+	+	+	–	+	+	+	–	

ment with oral contraceptives of the combination type although alterations, especially in the follicular development, are frequently found. It is generally agreed that the number of primordial and early primary follicles is mainly dependent on the age of the individual woman and, on the whole, seems to be independent both on the hormonal preparation used and the duration of therapy (e.g. 10 19 21 24). The discrepancies arise when the antral and Graafian follicles are considered In the present study we found typical antral and/or Graafian follicles in 5 out of 10 women after 6 to 72 months of treatment. This observation is in agreement with those of Maqueo et al. (10), Sánchez Rivera et al. (19) and Zañartu et al. (24) who all found equivalent follicles in some cases, even after long term treatment. In addition 2 out of the 10 women revealed a slight thickening of tunica albuginea, this finding confirms those in previous investigations (2, 10 18 21) On the other hand, we did not in any case find perifollicular fibrosis, which in some cases has been observed by other investigators (4 10 14 18, 25) during long term treatment with oral contraceptives. All the changes mentioned above seem to be reversible in the great majority of women as demonstrated previously by Lopez Llera et al. (7) and Weber (23) and now by us in the present study A few women, however develop secondary amenorrhoea after treatment has been discontinued. This amenorrhoea is usually caused by a functional disturbance in the hypothalamic pituitary system, but in some cases it might be explained by a marked fibrosis which fails to regress.

Goldzieher et al. (3) have reported that long-term treatment with oral contraceptives did not only cause an abolition of the midcycle peak of FSH and LH but did also significantly suppress the levels of both FSH and LH during the whole cycle. The suppression of plasma LH was more pronounced than that of plasma FSH. In addition they found that the values of both FSH and LH returned to normal levels during the first 3 months after cessation of treatment. These findings were entirely confirmed by the present investigation

It is still not known to which extent follicular growth and maturation depends upon stimulation with FSH and LH. Apparently a certain threshold level of serum FSH is necessary for the follicular development beyond the stage of early primary follicles, and it seems as if a certain threshold level of LH is required to obtain complete maturation and function of the Graafian follicle (1) This hypothesis was supported by the observations of Ross (17) and Sparkes et al. (20). They have studied a small group of women with the syndrome of olfacto-genital dysplasia. These women have a complete or partial agenesis of the olfactory lobes and a hypogonadotropic hypogonadism resulting in primary amenorrhoea. It was shown that the urinary excretion of both FSH and LH was very low and ovarian biopsies in these cases revealed only primordial and early primary follicles and a rare follicular cyst, but no antral or Graafian follicles. The threshold levels of FSH and LH which have to be exceeded to achieve development of a Graafian follicle must, however be rather low as we found antral and/or Graafian follicles in half of the women during long term treatment with oral contraceptives in spite of low values of both FSH and LH in serum.

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REFERENCES

1. Berger M. J. Taymor M. L., Karam, K. & Nudemberg, F. The relative roles of exogenous and endogenous follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in human follicular maturation and ovulation induction. Fertil Steril *23* 783 1972.
2. Diddle, A. W. Watts, G. F. Gardner W. H. & Williamson, P. J. Oral contraceptive medication. A prolonged experience. Amer J Obstet Gynecol *95* 489 1966.
3. Goldzieher J. W. Kleber J. W. Moses, L. E. & Rathmacher R. P. A cross-sectional study of plasma FSH and LH levels in women using sequential, combination or injectable steroid contraceptives over long periods of time. Contraception *2* 225 1970.
4. Grandesso, M. G. & Almeida, A. B. de Nor-esteróides anticoncepcionais. Estudo experimental baseado na histologia do útero, ovário e embrião. Rev Ginecol Obstet (Rio) *116* 108 1965.
5. Johannisson, E., Tillinger K. G. & Diczfalusy E. Effect of oral contraceptives on the ovarian reaction to human gonadotrophins in amenorrheic women. Fertil Steril *16* 292, 1965.
6. Lindhorst, C. A new progestogen-estrogen combination for gynecologic therapy and contraception. Int J Fertil *11* 35 1966.
7. Lopez-Llera, M. Alvarado-Duran, A. Rubach, L. & Carbia, E. Ovarian morphogenesis in the first spontaneous cycles after prolonged oral contraception. Int J Fertil *14* 162, 1969.
8. Ludwig, K. S. Zur funktionellen Morphologie des menschlichen Ovariums unter Einwirkung von Oestrogen-Gestagen-Kombinationspräparaten. Gynaekol Rundsch *6* 241, 1968.
9. Mall Haefeli, M. Ludwig, K. S. Kaiser M. & Cloeren, S. Beitrag zum Wirkungsmechanismus der oralen Ovulationshemmer beim Menschen. Gynaecologia (Basel) *160* 269 1965.
10. Maqueo, M. Rice-Wray E., Calderon, J. J. & Goldzieher J. W. Ovarian morphology after prolonged use of steroid contraceptive agents. Contraception *5* 177 1972.
11. Midgley A. R. Jr Radioimmunoassay: a method for human chorionic gonadotropin and human luteinizing hormone. Endocrinology *79* 10, 1966.
12. — Radioimmunoassay for human follicle-stimulating hormone. J Clin Endocrinol *27* 295 1967.
13. Midgley A. R. J. Rebar R. W & Niswender G. D. Radioimmunoassays employing double antibody techniques. Acta Endocrinol (Kbh) *63* (Suppl. *142*) 247 1969.
14. Plate, W. P. Ovarian changes after long-term oral contraception. Acta Endocrinol (Kbh) *55* 71 1967.
15. Pujol-Amat, P. Urgell-Roca, J. M., Esteban-Altirriba, J. & Márquez-Ramírez, M. Studies of ovarian biopsies from women cyclically treated with the combination ethynodiol diacetate + mestranol alone and together with human menopausal gonadotrophin (Pergonal) + HCG. Gynaecologia (Basel) *167* 237 1969.
16. Pujol-Amat, P. Urgell-Roca, J. M. & Márquez Ramírez, M. The ovarian response to gonadotrophins after administration of an oral contraceptive. J Obstet Gynaecol Br Commw *78* 261, 1971.
17. Ross, G. T. Gonadotropins and preantral follicular maturation in women. Acta Endocrinol (Kbh) in press.
18. Ryan, G. M., Jr Craig, J. & Reid, D. E., Histology of the uterus and ovaries after long-term cyclic norethynodrel therapy. Amer J Obstet Gynecol *90* 715 1964.
19. Sánchez-Rivera, G., Merlo, J. G., Escudero, M. & Botella-Llusiá, J. Action of several synthetic steroids of the human ovary. Amer J Obstet Gynecol *101* 665 1968.
20. Sparkes, R. J. Simpson, R. W. & Paulsen, C. A. Familial hypogonadotropic hypogonadism with anosmia. Arch Int Med *121* 534 1968.
21. Starup, J. The effect of gestagen and oestrogen treatment on the development of ovarian follicles: laparotomy observations. Acta Obstet Gynecol Scand *46* (Suppl. *9*) 15 1967.
22. Starup, J. & Østergaard, E. The mechanism in inhibition of ovulation in oral contraception II. The effect of PMS and HCG observed at laparotomy in patients treated with megestrol acetate together with mestranol. Acta Endocrinol (Kbh) *52* 292, 1966.
23. Weber W. Zur funktionellen Morphologie des menschlichen Ovariums nach Absetzen von Oestrogen-Gestagen-Kombinationspräparaten. Geburtsh Frauenheilk *29* 146, 1969.
24. Zañartu, J. Pupkin, M., Rosenberg, D. Guerrero, R. & Pupa, J. Estudio morfológico del ovario humano bajo el efecto de algunos esteroides y del dispositivo intrauterino usados para el control de la fertilidad. Rev Med Chil *95* 512, 1967.
25. Zussman, W. V. Forbes, D. A. & Carpenter R. J. Ovarian morphology following cyclic norethindrone-mestranol therapy. Amer J Obstet Gynecol *99* 99 1967.
26. Østergaard, E. L'aspect des ovaires au cours des traitements anti-ovulatoires. In L'inhibition de l'ovulation, Masson et Cie, Paris 1971, p. 157.

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MORPHOLOGY OF HUMAN OVARIES AFTER TREATMENT WITH AN INJECTABLE LONG-ACTING OESTROGEN PROGESTOGEN AGENT

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Abstract. Eight women of proven fertility received from 1–3 monthly injections each of Deladroxate®, a long-acting oestrogen-progestogen agent. The injections were administered on day 8 in the cycle, counted from first day of bleeding. Laparotomy with biopsies from both ovaries was performed on day 17–32 in the last treatment cycle. In 6 cases endometrial biopsies were also obtained. The numbers of primordial and secondary follicles were roughly estimated as normal. Maturing or mature follicles and follicular cysts were found in 5 of the patients. In one patient (with only one injection prior to laparotomy) a fresh corpus luteum was found. In one patient a slight degree of capsular fibrosis was seen, but stromal fibrosis was not seen in any case. Of the 6 endometrial biopsies obtained, 4 were of the secretory type, one was of the mixed phase type, and one was proliferative.

Since Pincus and co-workers published their results of large-scale oral contraceptive trials in Puerto Rico many reports have stated that different combinations of oestrogens and progestogens effectively inhibit conception. Nevertheless, the exact mechanism of action is not completely understood. The general concept is that ovulation is inhibited at the hypothalamo-pituitary level by interference with the production and/or secretion of luteinizing and follicle stimulating hormones, and it is further suggested that the anti-ovulatory steroids also exert a direct action on the ovaries by preventing follicular maturation. Several investigators have published reports dealing with the effect of different contraceptive agents on human ovarian morphology examined by direct inspection of the ovaries at laparotomy and by microscopy of ovarian biopsies (Table I). A rather consistent finding during long-term treatment is the presence of maturing or mature follicles and absence of fresh corpora lutea, but unfortunately only very few authors define the terms "maturing" and "mature" follicles. However if treatment is started relatively late in the cycle (8th day or later) and during the first treatment cycle, "escape ovulation" may take place (1, 3, 11, 13, 24, 31). Other characteristic patterns seem to be follicular atresia and diminished thecal proliferation (1, 3, 10), formation of follicular cysts (8, 9, 11, 22, 31), stromal fibrosis (14, 15, 22, 31) and thickening of the tunica albuginea (2, 14, 23). It should be mentioned, that Maqueo & Goldzieher (12) found cortical fibrosis in the ovaries in 11 out of 50 pregnant women at term, possibly as a result of the steroidal environment during pregnancy.

In previous reports (16, 17) the results of studies of long-term treatment with Deladroxate®, an injectable oestrogen-progestogen agent, have been published. Assessment of ovulation was carried out by means of basal body temperature recordings, endometrial biopsies, and pregnanediol and total pituitary gonadotrophin excretion, but none of the individual parameters used gave a 100% consistent picture. The present investigation was carried out, partly in an attempt to further elucidate the question of ovulation inhibition during treatment, partly in order to study the effect of Deladroxate® on ovarian morphology. Zañartu (28) and Zañartu et al. (29, 30) have described the effect on the ovaries of different other injectable contraceptive steroids (medroxyprogesterone acetate, chlormadinone acetate, and norethisterone oenanthate), but so far no studies of the effects of Deladroxate® on human ovaries have been published.

Østergaard & Starup (1968)	21	med. ac. mestr 100	(23–24	1–44	23–27	—		—		—
van Gemerenold & Ferin (1958)	3	17-methyl-B-nor-testosterone	5–23							
	9	'combined therapy		—	—	—	—	—	—	—
Zañartu et al. (1967)	7	sequential therapy'		—	—	—	—	—	—	—
Zañartu (1968)	18	inject. progestogens		—	—	—		—	—	—
	5	cl. ac.		—	—	—		—	—	—
Zañartu et al. (1970)	8	medroxyprogesterone-		2–6	—	—	—		+	—
	12	acetate 200–300 mg		7–14	—	—			+	—
Zussman et al. (1967)	18	nor-ethn. mestr 100	5–26	1 3	23–26	multi-cystic			+	—
Østergaard (1964)	33	meg. ac. mestr 100	5–24	1	24	—			—	—

Medroxyprogesterone acetate, cl. ac. chlormadinone acetate, nor-ethn. norethisterone, mestr 75 mestranol 0.075 mg, mestr 100 mestranol 0.10 mg, mestr 130 mestranol 0.13 mg, lyn. lynestrenol 2.5 mg, lyn. 5 lynestrenol 5.0 mg, nor-ethl. = norethynodrel, nor-eth. norethandrone, meg. ac. megestrol acetate, cl. ac. chlormadinone acetate, eth. diac. ethynodiol diacetate

MATERIAL AND METHOD

Eight women, ranging in age from 31 to 44 who had gained permission for surgical sterilization were included in the investigation. All the women were of proven fertility (previous deliveries, legal or spontaneous abortions). Each women received 1 3 injections of Deladroxate® combination of 150 mg 16α, 17α-dihydroxyprogesterone acetophenide and 10 mg oestradiol oenanthate in 1 ml of an oily solution, administered intramuscularly on the 8th (7th–9th) day of each cycle, counted from first day of bleeding. In the last treatment cycle, laparotomy was performed on day 17 32, and the ovaries were inspected and photographed, and biopsies were obtained from both ovaries by resection. All biopsies were cut and stained with haematoxylin and eosin and by the Van Gieson method. In 6 of the women endometrial biopsies were obtained in addition.

RESULTS

Table II summarises the results obtained by inspection and by examination of biopsies from the ovaries, and endometrial biopsies. The gross appearance of the ovaries was mostly characterised by the greyish-white smooth surfaces (Fig. 1). In one ovary a haemorrhagic follicular cyst of about 0.7 × 0.7 cm was visible on the surface (Fig. 1 patient no. 7).

Microscopy revealed normal stroma and capsules without fibrosis (Fig. 2) except for one case (patient no. 4) in which a slight to moderate degree of capsular fibrosis was seen (Fig. 3).

The numbers of primordial and secondary follicles (defined in accordance with the nomenclature of Watzka (27)) were roughly estimated as normal, but attempts to count them were not made since several of the biopsies were small, and the number of follicles may vary to a large extent from one site to another in the same ovary. In the ovaries from 5 of the 8 women, tertiary follicles and small follicular cysts were found (Fig. 4), and in one case (patient no. 6) a fresh corpus luteum was also found (Fig. 5). This was the only woman with only one injection prior to laparotomy.

Of 6 endometrial biopsies obtained on the day of laparotomy four were of the secretory type, one was of the mixed phase type, and one was purely proliferative.

DISCUSSION

It seems probable from the present findings that Deladroxate® does, to a great extent at least,

Table I Previous laparotomy studies of human ovaries during progestogenic and oestrogenic-progestogenic treatment

Reference	Number of laparotomy observations	Agents	Mode of treatment: Cyclically on days	Mode of treatment: Continuously	Number of treatment cycles (months) before laparotomy	Day of laparotomy	Ovarian surface	Fresh corp lutea	Maturing or mature follicles	Atretic follicles	Capsular fibrosis
Black & Govan (1968)	22	eth. diac. or eth. diac. + oestrogen	+ 5–25		one to several	various stages of cycle	—	+	+	+	—
Diddle et al. (1966)	1	nor-ethn + ethinyl oestradiol	+		61	mid-cycle	sclerotic	+	—	+	+
	2				46–62			—	—	+	+
Erb & Ludvig (1965)	2	lyn. + mestr 75	5–24		1–2	24–26	—	+	+	—	—
Garcia & David (1968)	31	nor-ethl. + mestr 100	5–25		—	latter half of cycle	—	—	+	+	—
Knuttson & al. (1965)	10	lyn. + mestr 75	4–24		1	25–27	normal	—	+	+	—
Kopera et al. (1964)	3	lyn. + mestr 75	5–25		—	—	—	—	—	—	—
	11	lyn.	5–25		—	—	—	—	—	—	—
Lauweryns & Ferin (1964)	3	lyn.		+	7–11	—	smooth	—	+	+	—
Linthorst (1966)	several	lyn. + mestr 75	+		1–2	—	—	—	+	—	—
	1	lyn. + mestr 75	+		2	10	—	+	+	—	—
Mall-Haefeli et al (1965)	5	lyn. + mestr 75; cl. ac. + mestr 75; nor-ethn. acetate + aethyloestradiol			2 12	15–23	smooth or polycystic	—	+	+	—
Matsumoto et al. (1960)	11	nortestosterone derivatives, or enovid	3 (7)–16		1	11 16	—	—	—	+	—
	7		7 (14)–23		1	12 24	—	+	+	—	—
Pincus (1956)	10	progesterone 300 mg	5–25		1	20–26	—	+	—	—	—
Plate (1968)	11	lyn. 5 + mestr 150; lyn. mestr 75; nor-ethn. acetate + ethinyl oestradiol; meg. ac. + ethinyl oestradiol; nor-ethn mestr 100	+		4–40	—	—	—	+	+	+
Pupa et al. (1967)	37	antiovulatory steroids	—		> 24	—	—	—	+	+	—
Pujol-Amat et al. (1967)	30	eth. diac. mest 100	—		1 21	20–27	—	—	—	—	—
Rock et al. (1957)	7	nor-ethn.	5–(20) 25		1 3	21 27–6 (next cycle)	sclerotic	—	+	—	—
Garcia et al. (1958)											
Rudel (1964)	5	nor-ethn		+	< 3	—	—	—	—	—	—
Ryan et al. (1964)	18	enovid 5 mg	5–24		< 24	—	—	—	—	+	—
Sørup (1967)	6	meg. ac.	5–24		1	24	normal				—
	3	mest 100	5–24		1	2	normal				—
	6	meg. ac. mest 100	5–24				normal	—		—	
	2		5–24		[illegible]	24	normal				

Table II. *Effects of Deladroxate® on ovaries and endometrium*

Subject	Number of injections before laparotomy	Day of laparotomy	Fresh corpora lutea	Capsular fibrosis	Atretic follicles	Follicular cysts	Endometrial biopsies*
1. U. N.	2	32	–	–	–	+	S
2. N. P.	3	17	–	–	+	–	S
3. E. L.	2	23	–	–	–	–	S
4. L. A.	2	21	–	(+)	–	+	S
5. R. P.	2	25	–	–	–	+	P (S)
6. L. C.	1	22	+	–	+	–	—
7. E. E.	3	23		–	–	+	—
8. I. H.	3	24	–	–	–	+	P

* S = secretory phase, P = proliferative phase.

exert its contraceptive effect by inhibiting ovulation, but it remains a question whether this inhibitory effect on ovulation is exerted via inhibition of the pituitary excretion of gonadotrophins, or whether it is due to direct effect on the ovary or both. Several authors (e.g. 25, 26) have made an attempt to answer the question by administering gonadotrophins during treatment with contraceptive steroids. The results, however, are somewhat confusing. In some investigations ovulation did take place when gonadotrophins were

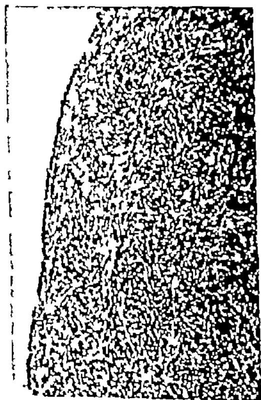
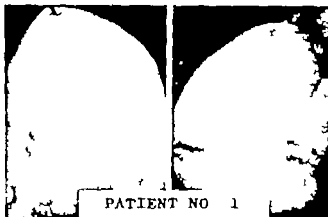
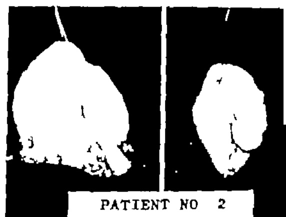


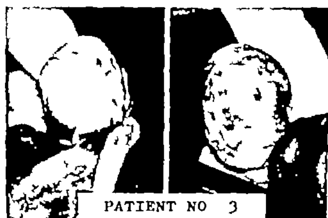
Fig. Ovarian biopsy from patient no. 7 with few primordial follicles and no secondary follicles. Haematoxylin-eosin stain, 100.



Fig. 3. Ovarian biopsy from patient no. 4 showing slight capsular fibrosis (upper part of picture). Van Gieson stain, 100.

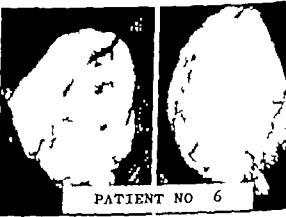












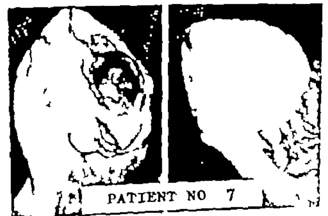




Fig. 1. Gross appearances of ovaries from 8 Deladroxate® treated women at laparotomy.



Fig. 5. Ovarian biopsy from patient no. 6 (one treatment cycle) showing fresh corpus luteum. Haematoxylin-eosin stain, × 40.

REFERENCES

1. Fuck, W. P. & Govan, A. D. T. The influence of contraceptive steroids on the structure and function of the Graafian follicle. J Obstet Gynaecol Br Commw 75: 1199, 1968.
2. Dobbs, A. W., Watts, G. F., Gardner, W. H. & Wilkinson, F. J. Oral contraceptive medication. A prolonged experience. Amer J Obstet Gynecol 95: 499, 1966.
3. Erb, H. & Ludwig, K. S. Corpus-luteum-Bildung während Einnahme eines hormonalen Antikonzeptivums. Gynaecologia (Basel) 159: 309, 1965.
4. van Gansewinkel, A. & Ferin, J. Les effects de la méthylœstrénolone sur l'ovaire humain. Bull Soc Roy Belge Gynecol Obstet 28: 442, 1958.
5. Garcia, C. R., Pincus, G. & Rock, J. Effects of three 19-nor steroids on human ovulation and menstruation. Am J Obstet Gynecol 75: 82, 1958.
6. Karlsson, F., Rybo, G. & Asberg, A. Anovulatory effect of lynestrenol in combination with mestranol. Acta Obstet Gynecol Scand 44: 325, 1965.
7. Kopera, H., Dukes, M. N. G. & Ipperman, G. L. Critical evaluation of clinical data on lyndiol. Int J Fertil 9: 69, 1964.
8. Lauweryns, J. & Ferin, J. Effects on the ovary of prolonged administration of lynestrenol: A histological study. Int J Fertil 9: 35, 1964.
9. Linthorst, G. The effect of oral contraception on the ovary. Conference, Scheveningen, The Netherlands, p. 95. Excerpta Medica Foundation 1966.
10. Mall-Haefeli, M., Ludwig, K. S., Keller, M. & Cloeren, S. Beitrag zum Wirkungsmechanismus der oralen Ovulationshemmer beim Menschen. Gynaecologia (Basel) 160: 269, 1965.
11. Matsumoto, S., Ito, T. & Inoue, S. Untersuchungen der ovulationshemmenden Wirkung von 19-Norsteroiden an laparotomierten Patientinnen. Geburtsh Frauenheilk 20: 250, 1960.
12. Maqueo, M. & Goldzieher, J. Hormone-induced alterations of ovarian morphology. Fertil Steril 17: 676, 1966.
13. Pincus, G.: Some effects of progesterone and related compounds upon reproduction and early development in mammals. Acta Endocrinol (Kbh), Suppl. 28, 1956.
14. Plate, W. P. Ovarian changes after long-term oral contraception. Acta Endocrinol (Kbh) 55: 71, 1967.
15. — Veranderingen in het Ovarium na langdurige orale Anticonceptie. Ned T Verlosk 68: 181, 1968.
16. Plesner, R. Contraception by an injectable long acting oestrogen-progestogen agent. Acta Endocrinol (Kbh) 61: 494, 1969.
17. — Ibid. III. Acta Endocrinol (Kbh) 69: 67, 1972.
18. Pega, J., Pupkin, M. & Garcia-Huidobro, M.: The mechanism of ovulation inhibition: A morpho-physiologic study. Med Gynaecol Sociol 3: 131, 1968.
19. Pujol-Amat, P., Urgell-Roca, J., Esteban-Altirriba, J., Marquez-Ramirez, M. & Hernández-Soler, J. Studies of ovarian biopsies from women cyclically treated with the combination ethynodiol diacetate and mestranol. Acta Endocrinol (Kbh), Suppl. 119, 1967.
20. Rock, J., Garcia, C. R. & Pincus, G. Synthetic progestins in the normal human menstrual cycle. Recent Progr Hormone Res 13: 323, 1957.
21. Rudel, H. El uso de progestágenos en el control de la ovulación humana. Second Annual Meeting of the Mexican Endocrine Society. Ixtapan de la Sal, Mexico 97, 1964.
22. Ryan, G. M., Craig, J. & Reid, D. E. Histology of the uterus and ovaries after long-term cyclic norethynodrel therapy. Amer J Obstet Gynecol 90: 715, 1964.
23. Starup, J. The effect of gestagen and oestrogen treatment on the development of ovarian follicles: laparotomy observations. Acta Obstet Gynecol Scand, Suppl. 9, 1967.
24. Starup, J. & Østergaard, E. Laparotomy observations during oral contraception. Acta Obstet Gynecol Scand 45, Suppl. 9, 1966.
25. — The mechanism in inhibition of ovulation in oral contraception. II. Acta Endocrinol (Kbh) 52: 292, 1966.
26. Taymor, M. L. & Rizkallah, T. Effect of norethindrone acetate upon gonadotrophin-induced ovarian function. J Clin Endocrinol 25: 843, 1965.
27. Watzka, M. Die Follikel. Handbuch der mikroskopischen Anatomie des Menschen, p. 79. Springer Verlag, Berlin 1957.



Fig. 4. Ovarian biopsies from patients nos. 4 and 5 with maturing follicles. Haematoxylin-eosin stain, × 40

administered, indicating a central effect on the pituitary; in other studies, no effect of gonadotrophins on ovulation was found indicating that inhibition was exerted directly on the ovarian level.

The sometimes pronounced stromal and capsular fibrosis, described by some authors (2, 14, 15, 23, 31) and haemorrhages in the theca interna (8, 14, 15) might be co-factors of importance in possible mechanisms acting directly on the ovarian level, but a majority of authors have not been able to find these changes, even during long term treatment with steroids, and as far as Deladroxate® is concerned, no such changes have been demonstrated except for one case, where a slight degree of capsular fibrosis was noted. Though the number of women examined in the present study is only small, it seems probable, therefore, that the effect of Deladroxate® on ovulation is primarily exerted via inhibition of pituitary gonadotrophin excretion. The presence of maturing (tertiary) follicles during treatment tempts one to assume that it may be the (...)cretion of luteinizing hormone which is inhibit(...) The assumption of a central inhibition is in agre(...)ment with the fact that in most cases treatme(...) with Deladroxate® suppressed urinary excreti(...) of total pituitary gonadotrophins (16, 17).

Previous investigations (17) have shown th(...) in the majority of cases the oestrogenic effe(...) of Deladroxate® on the endometrium predomi(...)nates over its progestogenic effect. However, (...) out of a total of 53 endometrial biopsies obtaine(...) during treatment with Deladroxate® were pure(...) secretory, a finding which is as difficult to ex(...)plain as is the finding in the present study of (...) secretory endometrial biopsies out of 6.

ACKNOWLEDGEMENTS

Deladroxate® was supplied by courtesy of E. R. Squibb & Sons. Ovarian and endometrial biopsies were examined by the Department of Pathology, Gentofte Hospital. The investigation was aided by a grant from the Ford Foundation.



Fig. 3. Ovarian biopsy from patient no. 6 (one treatment cycle) showing fresh corpus luteum. Haematoxylin-eosin stain, × 40

REFERENCES

1. Flack, W. P. & Govan, A. D. T. The influence of contraceptive steroids on the structure and function of the Graafian follicle. J Obstet Gynaecol Br Commw *75* 1199. 1968.
2. Dodds, A. W. Watts, G. P. Gardner, W. H. & Williamson, P. J. Oral contraceptive medication: A prolonged experience. Amer J Obstet Gynecol 95 499. 1966.

Erb, H. & Ludwig, K. S. Corpus-luteum-Bildung thrend Einnahme eines hormonalen Antikonzeptivums. Gynaecologia (Basel) *159* 309. 1965.

van Gansewinkel, A. & Ferin, J. Les effects de la méthyloestrénolone sur l'ovaire humain. Bull Soc Roy Belge Gynecol Obstet *28* 442, 1958.

Garcia, C.-R. Pincus, G. & Rock, J. Effects of three 19-nor steroids on human ovulation and menstruation. Am J Obstet Gynecol *75* 8.., 1958.

Kastrup, F. Ryba, G. & Asberg, A. Anovulatory effect of lynestrenol in combination with mestranol. Acta Obstet Gynecol Scand *44* 325, 1965.

Kopera, H. Dukes, M. N. G. & Ijzerman, G. L. Critical evaluation of clinical data on lyndiol. Int J Fertil 9 69. 1964.

8. Lauweryns, J. & Ferin, J.: Effects on the ovary of prolonged administration of lynestrenol: A histological study. Int J Fertil 9 35. 1964.
9. Linthorst, G. The effect of oral contraception on the ovary. Conference, Scheveningen, The Netherlands, p. 95. Excerpta Medica Foundation 1966.
10. Mall-Haefeli, M., Ludwig, K. S., Keller, M. & Cloeren, S. Beitrag zum Wirkungsmechanismus der oralen Ovulationshemmer beim Menschen. Gynaecologia (Basel) *160* 269. 1965.
11. Matsumoto, S. Ito, T. & Inoue, S. Untersuchungen der ovulationshemmenden Wirkung von 19-Norsteroiden an laparotomierten Patientinnen. Geburtsh Frauenheilk 20. 250. 1960.
12. Maqueo, M. & Goldzieher, J. Hormone-induced alterations of ovarian morphology. Fertil Steril *17* 676, 1966.
13. Pincus, G. Some effects of progesterone and related compounds upon reproduction and early development in mammals. Acta Endocrinol (Kbh), Suppl. *28*. 1956.
14. Plate, W. P. Ovarian changes after long-term oral contraception. Acta Endocrinol (Kbh) *55* 71. 1967.
15. — Veranderingen in het Ovarium na langdurige orale Anticonceptie. Ned T Verlosk 68. 181, 1968.
16. Plotnner, R. Contraception by an injectable long-acting oestrogen-progestogen agent. Acta Endocrinol (Kbh) *61* 494. 1969.
17. — Ibid. III. Acta Endocrinol (Kbh) *69*. 67. 1972.
18. Puga, J. Pupkin, M. & Garcia-Huidobro, M. The mechanism of ovulation inhibition: A morpho-physiologic study. Med Gynaecol Sociol *3* 131, 1968.
19. Pujol-Amat, P. Urgell-Roca, J. Esteban-Altirriba, J. Marques-Ramirez, M. & Hernández-Soler, J. Studies of ovarian biopsies from women cyclically treated with the combination ethynodiol diacetate and mestranol. Acta Endocrinol (Kbh), Suppl. *119*. 1967.
20. Rock, J. Garcia, C.-R. & Pincus, G. Synthetic progestins in the normal human menstrual cycle. Recent Progr Hormone Res *13* 323, 1957.
21. Rudel, H. El uso de progestagenos en el control de la ovulacion humana. Second Annual Meeting of the Mexican Endocrine Society. Ixtapan de la Sal, Mexico 97. 1964.
22. Ryan, G. M., Craig, J. & Reid, D. E. Histology of the uterus and ovaries after long-term cyclic norethynodrel therapy. Amer J Obstet Gynecol 90. 715, 1964.
23. Starup, J. The effect of gestagen and oestrogen treatment on the development of ovarian follicles: laparotomy observations. Acta Obstet Gynecol Scand, Suppl. *9*. 1967.
24. Starup, J. & Østergaard, E. Laparotomy observations during oral contraception. Acta Obstet Gynecol Scand *45*. Suppl. 9. 1966.
25. — The mechanism in inhibition of ovulation in oral contraception. II. Acta Endocrinol (Kbh) *53* 292, 1966.
26. Taymor, M. L. & Rizkallah, T. Effect of norethindrone acetate upon gonadotrophin-induced ovarian function. J Clin Endocrinol *25* 843, 1965.
27. Watzka, M. Die Follikel. Handbuch der mikroskopischen Anatomie des Menschen, p. 79. Springer Verlag, Berlin 1957.



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Previous investigations (17) have shown that in the majority of cases the oestrogenic effect of Deladroxate® on the endometrium predominates over its progestogenic effect. However 15 out of a total of 53 endometrial biopsies obtained during treatment with Deladroxate® were purely secretory a finding which is as difficult to explain as is the finding in the present study of 4 secretory endometrial biopsies out of 6.

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THE "PREGNANCY ZONE" PROTEIN AND ABORTION

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Abstract. The occurrence of the "pregnancy zone" protein was studied in the sera of 82 women with incomplete abortion and 272 women with apparently normal pregnancies undergoing induced abortion. In normal pregnancies the frequency of women showing the "pregnancy zone" protein increased from about 25% in gestation weeks 7–8 and to 69% in gestation weeks 17–18. In sera from women with incomplete abortion the "pregnancy zone" protein was found in a frequency of only 8–9% in gestation weeks 9–12. The results suggest that during pregnancy normal development of the ovum is necessary for the induction of the "pregnancy zone" protein. It is probable that women lacking this protein in gestation weeks 9–12 run a considerably higher risk of abortion than those who have developed this protein.

The "pregnancy zone" protein (PZ) is an α_2-globulin (7) which has been found in the serum of pregnant women (1, 2, 3, 7, 9, 11, 12, 13), women taking oral contraceptive drugs (4, 8, 9, 12), and also in men when treated with oestrogens for prostatic cancer (10, 11). PZ can be demonstrated in most pregnant women at term, but absence or low level of this protein has no apparent correlation with fetal welfare (2, 5, 7). So far no previous studies have been made of the relationship between spontaneous abortion and the occurrence of the PZ protein.

This study deals with the occurrence of the PZ protein in the sera of women with incomplete abortion and in women undergoing induced abortion.

MATERIAL AND METHODS

Serum samples were collected from two groups of women attending the Department of Obstetrics and Gynaecology, University of Umeå.

The first group consisted of 82 women with incomplete abortion. Evacuation of the uterus was performed in all cases. No febrile reaction was recorded. Since our interest was in early abortion, women with an estimated gestation length exceeding 20 weeks were excluded. The first serum sample was collected before evacuation.

The second group consisted of 272 consecutive women with apparently normal pregnancy undergoing induced abortion. The first serum sample was collected at the time of admission to the clinic.

In order to study the rate of disappearance of the PZ protein a second serum sample was collected from most of the women, 66 in the group of women with incomplete abortion and 204 in the group with induced abortion. The interval between the first and second serum sample varied between 1 and 4 weeks with a mean of 16 days. The serum samples were stored at −20°C until examination.

The occurrence of the PZ protein in the serum samples was tested by means of double diffusion in agar gel according to a method described in a previous publication (6). The precipitation reactions were recorded as "absent", "weak" or "strong". All samples were coded and tested blind. Information on gestation length (time interval since first day of last menstruation) and type of abortion (incomplete or induced) was obtained from the medical records after the completion of the laboratory investigations.

RESULTS

Tables I and II show the results for the series of induced and incomplete abortion respectively. The PZ protein was found more frequently ($P < 0.001$) among women undergoing induced abortion than in women with incomplete abortion.

In the series of induced abortion (Table I) the frequency of women showing the PZ protein, and the frequency of women showing a "strong" PZ reaction increased with gestation length. Between gestation lengths shorter than 9 weeks and longer than 14 weeks there were statistically significant differences with respect to PZ-positive women ($P < 0.001$) and women showing a "strong" PZ reaction ($P < 0.01$).

28 Zañartu, J Long-term contraceptive effect of injectable progestogens Inhibition and reestablishment of fertility Int J Fertil 13 415 1968
29 Zañartu, J Pupkin, M Rosenberg, D., Guerrero, R. & Puga J A.. A morphologic study of the human ovary under the influence of certain steroids, and of the intrauterine apparatus used for the control of fertility Acta Ginecol (Madr) 18 345 1967
30 Zañartu J Pupkin M Rosenberg, D Davanzens, A Guerrero, R., Rodriguez Bravo, R. & Garcia-Huidobro M Long-term effect of medroxyprogesterone acetate in human ovarian morphophysiology and sperm transport Fertil Steril 1 525 1970
31 Zussmann W Forbes, D A. & Carpenter R. J Jr Ovarian morphology following cyclic norethindrone-mestranol therapy Amer J Obstet Gynecol 99 99 1967
32 Østergaard, E. Inhibition of ovulation observed at laparotomy in patients treated with 6-dehydro-6-methyl-17α-acetoxyprogesterone. Int J Fertil 9 25 1964
33 Østergaard, E. & Starup, J Occurrence and function of corpora lutea during different forms of oral contraception. Acta Endocrinol (Kbh) 57 386 1968

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tude that it will not be influenced by a few wrongly classified cases. Factors like age, parity and number of previous abortions are not associated with the occurrence of the PZ protein at term of pregnancy (5) hence the correlation between spontaneous abortion and absence of PZ is unlikely to be spurious. Furthermore, no febrile reactions indicating criminal abortion were found among the women with incomplete abortion.

The results show that the PZ protein is found more rarely and in lower concentration in the serum of women with incomplete abortion than is that of women with "normal" pregnancy (to be legally aborted) of comparable gestation length. In the "normal" pregnancy group the concentration of the PZ protein increases significantly with gestation length. In the incomplete abortion group no such increase was found.

There are at least two alternative explanations of the low level of the PZ protein in the sera of women with incomplete abortion. 1) absence of the PZ protein is a cause of abortion, and 2) absence of the PZ protein is merely a sign which may indicate abortion. The second explanation seems more probable, since absence of the PZ protein is compatible with normal fetal development (5). About 10% of pregnant women at term lack this protein (5).

It is of interest that a certain fraction of women who take oral contraceptive drugs (8, 9) and males who are treated with oestrogens for prostatic cancer (10) also fail to develop the PZ protein in measurable amounts. The reason for this is not known, but it is possible that the PZ protein is not inducible in some individuals. The presence of the PZ protein may thus depend on two main factors (a) individual variations in inducibility and (b) the presence in sufficient amounts of an inducing agent. During early normal pregnancy such an inducing agent is apparently produced. Early fetal death or defects of the ovum are therefore a priori expected to lead to impaired development of the PZ protein. Thus women with incomplete abortion may lack the PZ protein because: 1) they belong to the non-inducible group of people 2) the inducing effect of normal pregnancy is lacking.

Three weeks after the induced abortion detectable PZ protein was found in 38% of the women examined. The rate of disappearance of

Table V *Occurrence of the PZ protein in the serum of 204 women before induced abortion and at variable time intervals after abortion*

Interval between blood samples (days)	Occurrence of PZ protein: Before abortion		After abortion		No. examined
		%		%	
9 and shorter	13	65	11	55	20
10–14	26	63	14	34	41
15–19	62	66	41	43	95
20 and longer	33	69	18	38	48
Total	134	66	84	41	204

PZ is evidently rather slow (Table V). This could imply that most women with early incomplete abortion never develop the PZ protein. In 3 women from both of the groups tested the pregnancy zone protein was demonstrated only in the second serum sample. This may of course, be due to failure of the method, but induction of the PZ protein has previously been reported to appear for the first time during labour and even in the puerperium (3).

Estimates were made of the risks of incomplete abortion in gestation weeks 9–12 for women with and without the PZ protein. The frequency of spontaneous abortion has been found to be 17.9% in retrospective studies of the population of northern Sweden (6). In the same population the frequency of spontaneous abortion in gestation weeks 9–12 has been estimated to about 12% (unpublished data). The following figures were used in the calculations: Frequency of pregnant women aborting in weeks 9–12 = 0.12, and of women not aborting in weeks 9–12 = 0.88. Frequency of PZ positive individuals = 0.08 among women with incomplete abortion in weeks 9–12 (Table II), and 0.60 among those not aborting in weeks 9–12 (Table I). For women who are PZ positive in weeks 9–12 the risk of aborting can be estimated as

$$R = \frac{0.12 \times 0.08}{0.12 \times 0.08 + 0.88 \times 0.60} = 0.015 \text{ and}$$

for women who are PZ-negative the risk of aborting can be estimated as

$$R = \frac{0.12 \times 0.92}{0.12 \times 0.92 + 0.88 \times 0.40} = 0.238$$

Table I *Occurrence of the PZ protein in the sera of 272 women before induced abortion*

Gestation length (weeks)	n	PZ protein Absent	Weak	Strong	% Present	% Strong
7–8	16	12	4	0	25	0
9–10	96	43	40	13	55	14
11–12	107	38	46	23	64	21
13–14	12	5	4	3	58	25
15–16	17	3	6	8	82	47
17–18	17	2	9	6	88	35
19–20	4	1	2	1	—	—
21 22	1	1	0	0	—	—
23–24	2	0	0	2	—	—
Total	272	105	111	56	61	21

In the series of incomplete abortion (Table II) no time trend was found. The lowest frequency (8–9%) was found in weeks 9–12. Furthermore strong" PZ reactions with one exception were lacking in this group.

A second serum sample was obtained from 204 of the women after induced abortion and from 66 women after evacuation for incomplete abortion. The mean time interval between the first and second samples was 16 7 days for induced abortion and 15 0 days for incomplete abortions.

The occurrence of the PZ protein in the first and second samples is shown in Tables III and IV for induced and incomplete abortion respectively Of the 134 women who were PZ-positive before induced abortion 81 showed the PZ protein also in the second sample (Table III). This represents a 40% decrease in the number of PZ

Table II *Occurrence of the PZ protein in the sera of 82 women with the diagnosis of incomplete abortion before evacuation*

Gestation length (weeks)	n	PZ protein Absent	Weak	Strong	% Present	% Strong
5–6	2	2	0	0	—	—
7 8	12	8	3	1	33	8
9–10	26	24	2	0	8	0
11 12	33	30	3	0	9	0
13–14	7	6	1	0	14	0
15–16	2	1	1	0	—	—
Total	82	71	10	1	13	1

Table III *Occurrence of the PZ protein in the sera of 204 women before induced abortion and 1–4 weeks after*

PZ(+) = PZ protein present PZ(−) = PZ protein absent

		No. examined	1–4 weeks after abortion PZ(+)	PZ( )
Before abortion	PZ(+)	134	81	53
	PZ(−)	70	3	67
Total		204	84	120

positive" women during a period of approximately 16 days.

In the group of incomplete abortion (Table IV) 7 women were PZ-positive in the first sample and 3 of them remained PZ positive in the second sample.

In both groups 3 women who were negative for the PZ protein in the first sample were found to be PZ positive in the second sample.

Table V shows the change in frequency of detectable PZ protein between the first and the second serum samples in relation to different time intervals. The incomplete abortion group was too small to allow a similar analysis.

DISCUSSION

The present results are based on comparisons of two different groups of women with incomplete abortion and normal" pregnancy respectively In a small number of cases the diagnosis may of course have been inaccurate. The difference between the groups is, however of such magni-

Table IV *Occurrence of the PZ protein in the sera of 66 women with the diagnosis of incomplete abortion before evacuation and 1–4 weeks after*

PZ(+) PZ protein present PZ(−) PZ protein absent

		No examined	1–4 weeks after abortion PZ( )	PZ( )
Time of abortion	PZ(+)	7	3	4
	PZ(−)	59	3	56
Total		66	6	60

tude that it will not be influenced by a few wrongly classified cases. Factors like age, parity and number of previous abortions are not associated with the occurrence of the PZ protein at term of pregnancy (5), hence the correlation between spontaneous abortion and absence of PZ is unlikely to be spurious. Furthermore no febrile reactions indicating criminal abortion were found among the women with incomplete abortion.

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Table V. *Occurrence of the PZ protein in the serum of 204 women before induced abortion and at variable time intervals after abortion*

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	Before abortion		After abortion		
		%		%	
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PZ is evidently rather slow (Table V). This could imply that most women with early incomplete abortion never develop the PZ protein. In 3 women from both of the groups tested the pregnancy zone protein was demonstrated only in the second serum sample. This may of course, be due to failure of the method, but induction of the PZ protein has previously been reported to appear for the first time during labour and even in the puerperium (3).

Estimates were made of the risks of incomplete abortion in gestation weeks 9–12 for women with and without the PZ protein. The frequency of spontaneous abortion has been found to be 17.9% in retrospective studies of the population of northern Sweden (6). In the same population the frequency of spontaneous abortion in gestation weeks 9–12 has been estimated to about 12% (unpublished data). The following figures were used in the calculations: Frequency of pregnant women aborting in weeks 9–12 = 0.12, and of women not aborting in weeks, 9–12 = 0.88. Frequency of PZ positive individuals = 0.08 among women with incomplete abortion in weeks 9–12 (Table II), and 0.60 among those not aborting in weeks 9–12 (Table I). For women who are PZ positive in weeks 9–12 the risk of aborting can be estimated as:

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for women who are PZ-negative the risk of aborting can be estimated as:

$$R = \frac{0.12 \times 0.92}{0.12 \times 0.92 + 0.88 \times 0.40} = 0.238$$

Thus the risk of abortion in weeks 9–12 may be about 16 times higher for women lacking the PZ protein than for those who have this protein The estimated risk figures are of course subject to statistical and other errors and should therefore be interpreted with caution. They indicate however that PZ determinations in early pregnancy may be of predictive value and that the presence of a strong PZ protein in gestation weeks 9–12 is a good indicator of a normally progressing pregnancy

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REFERENCES

1 Afonso J F & de Alvarez, R. R. Amer J Obstet Gynecol *86* 815 1963
2. — Amer J Obstet Gynecol *89* 204, 1964.
3 Afonso, J F & Farnham, N G Amer J Obstet Gynecol *84* 199 1962.
4 de Alvarez, R. R. & Afonso, J F Penn Med J *70* 43 1967
5 Beckman, G von Schoultz, B. & Stigbrand, T Acta Obstet Gynecol Scand (in press), 1973.
6. Beckman, L., Beckman, G & Magnusson, S S Hum Hered *22* 15 1972.
7 Beckman, L., Beckman, G & Stigbrand, T Hum Hered *20* 530 1970
8 Beckman, L., von Schoultz, B. & Stigbrand, T Acta Obstet Gynecol Scand *50* 369 1971
9 — Acta Obstet Gynecol Scand *52* 157 1973
10. — Urol Res *1* 67 1973
11 Cooper D W Nature 200 892, 1963
12. Margolis, J & Kendrick K. G Aust J exp Biol med Sci *47* 637 1969
13 Smithies, O Advances Protein Chem *14* 65 1959

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ENDOCRINOLOGICAL ASPECTS OF MALE INFERTILITY

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Abstract In order to clarify to what extent hormonal disturbances may cause infertility in men, a study was made of 93 infertile men aged 23–52 years. Their spermiograms was defective. Urinary excretion of total gonadotropins, LH, estrogens, 17-ketogenic steroids, 17 ketosteroids and fractionated 17-ketosteroids was determined. The patient material was divided into 4 groups according to the excretion of total gonadotropins and estrogens in the urine. The groups were compared with a control group of 50 healthy males (aged 16–47 years) of proven fertility.

Group I had an elevated excretion of total gonadotropins and estrogens in the urine. No treatment was possible in this group. In group II, the excretion of total gonadotropins was <40 MU/litre, the excretion of LH was slightly elevated and the excretion of estrogens in the urine was higher than in the control group. A faulty testicular response to normal gonadotropin stimulation or an imbalance in the FSH/LH ratio might explain the increased estrogen excretion in the urine. In group III, the excretion of total gonadotropins and estrogens in the urine was normal and the excretion of 17-ketogenic steroids was slightly diminished.

In group IV there was a decreased excretion of steroids in the urine, which may support the diagnosis of pituitary hypofunction. This group is the only one in which treatment with gonadotropins might prove successful. The excretion of DHA was low in groups I and IV probably depending on the condition of the germinal epithelium.

The conclusion is reached that the cause of infertility in males may occasionally be a hormonal disturbance depending on hypothalamus-pituitary hypofunction. Only men who have a low excretion of gonadotropins and steroids in the urine may have a chance of becoming fertile after treatment with gonadotropins or releasing hormones.

Endocrinological disorders in males such as pituitary hypofunction and faulty steroid biosynthesis in the testis, may result in deficient spermatogenesis and infertility. An investigation of the hormonal activity in infertile men, studied by means of determination of gonadotropins and steroids excreted in the urine, has therefore been carried out. A careful examination of the spermatozoal morphology has also been made. MacLeod (11) points out that a careful differentiation between mature and immature sperm cells in the seminal analysis can replace a testis biopsy. From the amount of 17-ketosteroids excreted in the urine, no conclusion concerning the testosterone production in the testes can be made. Only 30% originates from the testes and the rest from the adrenals. Because of the metabolic interconversion between the testicular and adrenal precursors to 17-ketosteroids the origin of any given urinary 17-ketosteroid is lost. Determination of fractionated 17 ketosteroid in the urine for the same reason usually gives no better information. Birke et al (1) and Johnsen (8) have found that the amount of androgen excreted in the urine decreases with increasing age. Young men have often a high excretion of dehydroepiandrosterone. Johnsen (9) found a normal pattern of urinary excretion of fractionated 17-ketosteroids in 3 infertile men. Furuhjelm et al. (4) confirmed this observation on a larger series.

The amount of estrogens excreted in the urine reflects the Leydig cell function. Eighty per cent of the biologically active estrogens in the urine originate from the testes. Determination of the estrogen excretion in the urine is therefore valuable. Determination of testosterone in plasma and in urine is also of great value in the evaluation of the function of the Leydig cells.

MATERIAL

In a study on 67 men with deficient spermatogenesis and infertility reported by us at the Sixth Congress on

Table I *The hormonal excretion in normal and infertile men*

	Fertile men $N=50$	Group I $N=6$	Group II $N=39$	Group III $N=24$	Group IV $N=29$
Estrogens, μg/24 hrs	5.0±0.2	9.0±0.8	6.7±0.5	4.4±0.2	2.3±0.2
17 ketogenic steroids, mg/24 hrs	9.7±0.6	10.0±1.0	10.0±0.6	8.8±0.5	7.5±0.4
17 ketosteroids, mg/24 hrs	11.5±0.5	8.7±0.8	10.5±0.4	10.5±0.7	8.1±0.5
Fractionated 17 KS, mg/24 hrs	8.0±0.6	4.7±0.7	8.2±0.6	8.8±0.5	5.5±0.5
Dehydroepiandrosterone, mg/24 hrs	1.1±0.2	0.1±0.1	1.2±0.3	1.1±0.3	0.5±0.2
Androsterone mg/24 hrs	4.2±0.3	2.1±0.4	4.4±0.3	5.1±0.4	3.1±0.3
Etiocholanolone mg/24 hrs	2.7±0.3	2.5±0.4	2.5±0.2	2.5±0.3	1.8±0.3
LH IU/24 hrs			51±5.7		79±6.3

Sterility and Infertility in Tel Aviv 1968 the urinary excretion of gonadotropins and steroids was determined (5). This study has been extended to include an additional 98 subjects aged 23–57 years where the female partner was estimated to be fertile. Due to methodological changes the two groups cannot be combined. Fifty healthy males aged 16–47 years acted as controls. Most of them were fathers of newly-born babies delivered in our department and the others had normal sperm counts. In the group of infertile men the seminal analysis was defective with abnormal sperm cells exceeding 50% and/or a low concentration of sperm cells, less than 20 million/ml. All the seminal analyses were examined by one of us (B Jonson). No men with varicocele were included in the series. The excretion in the urine of total gonadotropins, LH, estrogens, 17 ketogenic steroids, 17-ketosteroids and fractionated 11-deoxy 17-ketosteroids was determined.

METHODS

Total gonadotropins were extracted according to the method of Johnsen (8). Instead of using the weight of the uterus as the parameter for quantitative determination we examined follicle maturation in the ovaries as described by Hamburger (7). LH was determined by a radio-immunological method of Wide & Porath (13). The estrogens determined included estradiol and estrone only and the method of Carlstrom & Furuhjelm (3) was used. 17 ketosteroids were determined according to Vestergaard (1) and fractionated 11-deoxy 17-ketosteroids by the method of Carlström et al. (2)

Table II *Seminal analysis—sperm quality in groups I–IV*

Group	Volume (ml)	% motility after 2 hrs	millions sperm cells/ml	abnormal sperm cells
I	4.1±0.5	36±8.9	36± 3.4	78±5.9
II	4.7±0.3	36±3.0	56± 7.4	73.1±1.8
III[b]	3.8±0.2	39±5.1	76±10.6	71.6±2.3
IV[a]	3.5±0.3	28±3.2	55±10.4	76.7±2.0

3 cases of aspermia.
[b] 1 case of aspermia.
3 cases of aspermia.

RESULTS

The results are listed in Table I. In the fertile male subjects the total gonadotropin excretion was less than 40 MU/l of urine in all cases. The excretion of estrogens was 5.0±0.2. The infertile men were divided into four groups according to the excretion of gonadotropins and estrogens.

Group I comprises 6 males with an urinary excretion of total gonadotropins over 40 MU/litre of urine.

Group II comprises 39 males with an urinary excretion of total gonadotropins of 10–40 MU/litre of urine or less than 10 MU/litre but with an excretion of estrogens in the urine of more than 6 μg/24 hours, indicating an adequate gonadotropic stimulation. The excretion of LH was also determined. The mean value for the LH excretion was *51±5.7 IE/24 hours*.

Group III comprises 24 males who had an urinary excretion of total gonadotropins less than 10 MU/24 hours and an excretion of estrogens of 6–4 μg/24 hours.

Group IV comprises 29 males who had an urinary excretion of total gonadotropins less than 10 MU/litre and an excretion of estrogens less than 4 μg/24 hours.

The mean excretion of estrogens was high in group I i.e. 9.0±0.8 μg/24 h. In comparison with the control group the excretion is higher ($P<0.01$). In group II the mean excretion of estrogens was somewhat higher than in the control group ($P<0.01$). In group IV the mean excretion of estrogens was 2.3±0.2 μg/24 h i.e. lower than in the control group $P<0.001$)

The mean excretion of 17-ketogenic steroids in groups I and II does not differ from the excretion in the control group. In group III the mean excretion is somewhat lower than in the control group, and also lower in group IV than in the control group ($P < 0.01$). The mean excretion of 17-ketosteroids is lower than in the control group in group IV ($P < 0.001$). The mean excretion of DHA is lower in groups I and IV than in the control group ($P < 0.05$ and $P < 0.01$ respectively).

DISCUSSION

In group I where the gonadotropin excretion is elevated we find a higher estrogen excretion than in the control group. This is in agreement with the fact that increased gonadotropin secretion stimulates steroid biosynthesis in the Leydig cells. Testicular biopsy was made in 5 of 6 cases and showed inhibition of spermatogenesis and an abundance of Leydig cells. The number of sperm cells is low: 3 cases have azoospermia. Our observation of subjects with poorly developed spermatogenesis and elevated urinary excretion of gonadotropins support Johnsen's theory (10) that the last stage of spermatogenesis involving maturation of spermatozoa liberates substances involved in the testicular-hypothalamic feed-back mechanism. W conclude that patients with high excretion of gonadotropins in the urine are not amenable to treatment due to the damage to their germinal epithelium.

The males in group II have a higher excretion of estrogens than those in the control group. The increased estrogen secretion from the testis may depend either on a faulty testicular response to normal gonadotropin stimulation or an imbalance in the FSH/LH ratio resulting in an increased excretion of estrogens. The mean excretion of LH in this group is 55 ± 5.7 IU/24 h, a fairly high value. Increased gonadotropic stimulation is therefore possible explanation of the increased excretion of estrogens.

The quality of the sperm in the different groups is listed in Table II. There are no great differences between the different groups. The percentage of abnormal sperm cells is somewhat higher in groups I and IV than in the others. In group I which includes 6 cases, there are three cases of azoospermia. The motility of the sperm cells is low in group IV only 28 % after 2 h. The decreased motility may be a sign of deficient hormonal stimulation as group IV has a low excretion of steroids in the urine. There is reason to believe that hypofunction in the hypothalamic-pituitary system could be the cause of infertility in this group as the low excretion of estrogens indicates incomplete gonadotropic stimulation of the testes.

The determination of fractionated 17-ketosteroids in infertile males has hitherto proved to be of little diagnostic aid. It is therefore of some interest that the excretion of DHA is low in groups I and IV. Very low excretion of DHA is found in women suffering from long-standing amenorrhea after taking contraceptive pills, in whom no growth of follicles in the ovaries is found (6). The secretion of DHA may have some connection with the condition of the germinal epithelium. More investigations in this field are needed, however, before definite conclusions can be made.

From a comparison between the seminal analysis and the hormone excretion in the different groups it is evident that the cases in group IV are those where a defective function of the hypothalamic-pituitary system may indicate treatment with gonadotropins or releasing hormone. Probably however not all of them are suitable for hormone therapy. Determinations of FSH, LH and testosterone in plasma will surely facilitate the selection of suitable cases.

REFERENCES

1. Birke, G., Diczfalusy, E. & Plantin, N. O., Assessment of the functional capacity of the adrenal cortex. I. Establishment of normal values. J Clin Endocrinol 18, 736, 1958.
2. Carlström, K., Furuhjelm, M., Ingelman-Sundberg, A. & Lunell, N. O.: Gas chromatographic estimation of individual urinary 11-deoxy-17-ketosteroids. Int J Gynaecol Obstet 7 124 1969.
3. Carlström, K. & Furuhjelm, M.: A routine clinical method for the estimation of low polar oestrogens in human urine. Acta Obstet Gynecol Scand 50 259 1971.
4. Furuhjelm, M., Jonson, B. & Lagergren, C. G.: Pattern of 17-ketosteroid urinary excretion in infertile men. Acta Endocrinol (Copenhagen) 42 97 1963.
5. Furuhjelm, M., Carlström, K. & Enbörning, G.: Endocrinological aspects of male infertility. Proc Sixth World Congress on Fertility and Sterility. Tel Aviv 1968 259 1970.

6. Furuhjelm M & Carlström, K. Amenorrhoea following use of combined contraceptives. Acta Obstet Gynecol Scand. In press.
7. Hamburger C. Studies on Gonadotropic Hormones. Levin & Munksgaard Copenhagen, 1933
8. Johnsen, S G A clinical routine-method for the quantitative determination of gonadotropins in 24-hour urine samples. Acta Endocrinol (Copenhagen) *28* 69 1958
9. — Studies on the testicular hypophyseal feed-back mechanism in man. Acta Endocrinol (Copenhagen) Supp *90* 99 1964
10. — The stage of spermatogenesis involved in the testicular-hypophyseal feed-back mechanism in man. Acta Endocrinol (Copenhagen) *64* 193 1970
11. MacLeod J A., Pazianos, A. S. & Ray B The restoration of human spermatogenesis and of the reproductive tract with urinary gonadotrophs following hypophysectomy Fertil Steril *17* 7 1966
12. Vestergaard, P Rapid micro-modification of the Zimmerman/Callow procedure for the determination of 17 ketosteroids in urine. Acta Endocrinol (Copenhagen) *8* 193 1951
13. Wide, L. & Porath, J Radioimmunoassay of proteins with the use of Sephadexcoupled antibodies Biochem Biophys Acta *130* 257 1966

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CASE REPORT

COINCIDENCE OF GRANULOSA-CELL TUMOUR OF OVARY AND DEVELOPMENT OF CARCINOMA IN RECTAL ENDOMETRIOSIS

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Abstract. A case is reported of the co-existence of granulosa-cell tumour in the ovary and endometrial cancer of the rectum in a 36-year-old woman. The endometrial cancer originated in the endometriotic tissue of the muscular layer of the rectum. Ovarian and pelvic endometriosis together with endometriosis of the small intestine had been diagnosed earlier. The patient had presented symptoms of endometriosis for several years. The simultaneous occurrence of granulosa-cell tumour and malignant transformation of endometriotic tissue is considered by the author as probably having a causal background, analogous to the development of hyperplasia of uterine endometrium or carcinoma of the endometrium in patients with hormone producing granulosa-cell tumours.

According to Clemmesen (3) the risk of a person who already has a malignant tumour developing a new malignant tumour is no greater than the risk in non-affected persons.

Moertel (16) concludes that there is no evidence to suggest that malignant disease of an estrogen sensitive organ increases the possibility of malignancy in other estrogen sensitive organs. However, there is a possible and rare exception in women with functionally active ovarian neoplasms, where increased and prolonged estrogen stimulation is considered to play some part in the development of uterine endometrial carcinoma.

A case is reported in the present work in which two rare malignant tumours occurred at the same time. The above mentioned theory of the origin of the two tumours is considered most probable.

CASE HISTORY

A 36-year-old woman who had had regular menstruation from the age of 13, a normal birth when 24 years old and a spontaneous abortion one year later. Sterile since. Hysterosalpingography in 1963 showed no abnormality.

In 1970 laparotomy with right salpingo-oophorectomy and left salpingostomy was performed in the gynecological department. The right ovary was polycystic with several blood-filled cysts. Several endometrioma-like infiltrations could be seen behind the uterus, in the bottom of the pouch of Douglas and on a couple of loops of small intestine adhering to the walls of the pouch of Douglas. The left ovary was normal.

Microscopic examination showed endometriosis both in the right ovary and in the tissue removed from the pouch of Douglas.

The patient was plagued by symptoms of pelvic endometriosis for the following two years and was therefore readmitted to hospital. Physical examination at that time revealed an irregular solid tumour posteriorly and to the left of the uterus attached to the surrounding tissue. Sigmoidoscopy showed normal mucosa with a bend at the recto-sigmoid junction. Barium enema demonstrated, in agreement with this, a constriction of several centimetres in length.

Her menstruation was still regular. Hormone analysis was not performed as it did not appear to be indicated.

Soon afterwards the patient was subjected, in the department of surgery, to laparotomy with hysterectomy and left salpingo-oophorectomy and resection of the rectum. A left adnexal tumour was found at operation,

6. Furuhjelm M & Carlstrom, K.. Amenorrhoea following use of combined contraceptives. Acta Obstet Gynecol Scand. In press.
7 Hamburger C. Studies on Gonadotropic Hormones. Levin & Munksgaard, Copenhagen 1933
8 Johnsen, S G A clinical routine-method for the quantitative determination of gonadotropins in 4-hour urine samples. Acta Endocrinol (Copenhagen) *28* 69 1958
9 — Studies on the testicular-hypophyseal feed-back mechanism in man. Acta Endocrinol (Copenhagen). Supp. *90* 99 1964
10 — The stage of spermatogenesis involved in the testicular-hypophyseal feed-back mechanism in man. Acta Endocrinol (Copenhagen) *64* 193 1970
11 MacLeod J A., Pazianos, A. S. & Ray B The restoration of human spermatogenesis and of the reproductive tract with urinary gonadotropins following hypophysectomy Fertil Steril *17* 7 1966.
12 Vestergaard, P Rapid micro-modification of the Zimmerman/Callow procedure for the determination of 17 ketosteroids in urine Acta Endocrinol (Copenhagen) *8* 193 1951
13 Wide L. & Porath, J Radioimmunoassay of proteins with the use of Sephadexcoupled antibodies. Biochim Biophys Acta *130* 257 1966.

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Autopsy

Recurrent tumour formation was found in the left side of the pelvis at autopsy There were metastases along the aorta and tumour conglomerates in a position corresponding to the lymph glands of Virchow in the left supraclavicular fossa. In addition metastases were found by microscopy in the lungs and pleura. The liver was considerably enlarged and more than 4/5 consisted of tumour tissue. The tumour tissue was seen on microscopy to be built up of the same components as those demonstrated in the granulosa-cell tumour found at operation. Neither macroscopic examination nor serial sections showed tumour tissue in the rectum.

DISCUSSION

Ten to twenty per cent of women in the fertile age are presumed to suffer from endometriosis (12). The ovaries are the most frequent site of endometriosis after the uterus and thereafter follow the peritoneum in the pelvis and the intestines (15).



Fig. 3. The endometrial cyst, the one half of which is smooth-walled and the other half showing malignant transformation x approx. 60



Fig. 4 Greater enlargement of the smooth-walled part of Fig. 3 approx. 540.

Malignant transformation of endometriosis was first described by Sampson in 1925 (19). However the case reported by Sampson was not convincing, on the other hand, those reported by Tellum in 1946 were (21) Grey published the 28th case in 1967 (10)

Both Ridley (18) and Crist (4) pointed out that more cases probably occur as malignancy cannot be demonstrated with certainty as originating from the endometriosis because it must be presumed that this tissue is destroyed by the malignant tumour I addition extrauterine pelvic neoplasms are rarely diagnosed early owing to the lack of symptoms.

Sampson (19) suggested the criteria which must

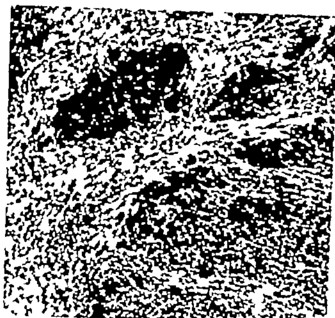


Fig 1 The granulosa-cell tumour with cellular poly morphism and mitoses. × approx. 105

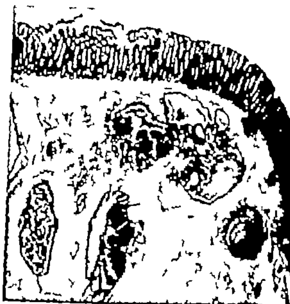


Fig 2 General view of the rectum. The muscular layer and submucosa are the site of infiltration of the endometrial carcinoma × approx. 10.

together with a slightly enlarged uterus, which was pushed to the right by a large mass in the pouch of Douglas. Large quantities of chocolate-like fluid were removed from this site An infiltrating process, the diameter of which was approximately 2 / cm was felt in the anterior wall of the rectum. There was no ascites and no abdominal or liver metastases.

The patient was given radiation treatment postoperatively Death occurred four months after the operation.

PATHOLOGY

Surgical specimen

The uterus was 7 cm long The cervix, endometrium and myometrium were normal macroscopically

The left ovary measured 4 × 5 × 6 cm, was well defined with a mottled brownish-grey surface and on cutting had a similar appearance Well defined smooth walled cysts were found when the organ was sliced. Its consistency was firm but elastic.

Microscopy of the uterus showed a somewhat hyperplastic endometrium in the secretory phase. Follicular cysts and a corpus luteum were seen in the ovary together with endometrial cysts and tumour tissue consisting of broad solid tumour cell groups or thin cords. In places there were Call–Exner bodies. The cells were relatively small of variable size and with ill-defined cell borders, sparse cytoplasm considerable nuclear polymorphism and innumerable mitoses (Fig. 1). Infiltrative growth of the tumour was found in the wall of the fallopian tube.

Histological diagnosis. granulosa-cell tumour of the ovary

The rectum was covered throughout with normal mucosa. Below the mucosa there was greyish-brown tissue having no well defined limits. The rectal mucosa was normal. However microscopy revealed a few diverticula. In the submucosa, muscle layer and adventitia single or small groups of gland lumina were found which could be very dilated. They were surrounded by endometrial-like stromal cells or fibrous stroma with macrophages containing pigment (Figs. 2 and 3). The glands were either smoothed walled (Fig. 4), covered with one or several layers of cylindrical epithelium, or papillary-like projections could divide the glands, and the epithelium was then of several layers with moderate cellular polymorphism and a few mitoses (Fig. 5). Squamous cell metaplasia was found occasionally There were no clear cells or cells containing mucus, either with periodide-Schiff (PAS), Best's carmine mucicarmine or alcian blue pH 2.5 (Mowry) staining. However mucus was seen in the lumina of a few glands.

Histological diagnosis endometrial carcinoma of the rectum.

REFERENCES

1 Aaberg, C.: Studier over endometriosens klinik, p. 210. Munksgaard, Copenhagen, 1944.
2 Busby T & Anderson, G W Feminizing mesenchymomas of the ovary Am J Obstet Gynecol *68* 1391, 1954
3 Clemmesen, J Statistical studies in malignant neoplasms, p 29 Munksgaard, Copenhagen, 1965
4 Crin, T Brunner W E. & Edwards, J R.: Adenoacanthoma originating in endometriosis of the ovary Obstet Gynecol *37* 419 1971
5 Diddle, A. W Granulosa and theca cell ovarian tumors: prognosis Cancer *5* 215, 1952.
6 Dockerty M. B Pratt, J H. & Decker D G: Primary adenocarcinoma of the rectovaginal septum probably arising from endometriosis. Cancer *7* 893 1954
7 Emge, L Endometrial cancer and feminizing tumors of the ovaries. Obstet Gynecol *1* 511, 1953
8 Fathalla, M F Malignant transformation in ovarian endometriosis. J Obstet Gynaecol Br Commw *74* 85 1967
9 Ferreira, H P & Clayton, S G. Three cases of malignant change in endometriosis, including two cases arising in the recto-vaginal septum J Obstet Gynaecol Br Commw *65* 41, 1958.
10 Gray L A. & Barnes, M. L.: Endometrioid carcinoma of the ovary Obstet Gynecol *29* 694 1967
11 Jacobsen, B. B. & Herting, S. C.: Endometriosis coli sigmoidei Ugeskr Laeger *134* 541 1972.
12 Jenkinson, E. L. & Brown, W H Endometriosis. JAMA *122* 349 1943
13 Lauslahti, Kalevi: Malignant external endometriosis. Acta Pathol Microbiol Scand, Sect. A, Suppl. 233: 98, 1972.
14 Mansell, H. & Hertig, A. T Granulosa-theca cell tumors. Obstet Gynecol *6* 385 1955
15 Masson, J C.: Surgical significance of endometriosis. Ann Surg *102* 819 1935.
16. Moertel, C. G Multiple primary malignant neoplasms, their incidence and significance. Recent Results in Cancer Research, vol. 7 p. 22, Springer Verlag, Berlin, Heidelberg and New York, 1966
17 Plata, W P Clear-cell adenocarcinoma in an endometrioid ovarian cyst. Obstet Gynecol 27 428 1967
18. Ridley J H Primary adenocarcinoma in implant of endometriosis. Obstet Gynecol 27 261 1966.
19 Sampson, J A.: Endometrial carcinoma in ovary arising in endometrial tissue in that organ. Arch Surg *10* 1 1925
20. Scott, R. B Malignant changes in endometriosis. Obstet Gynecol 2. 283, 1953
21 Tellem, G.: Carcinoma arising in ovarian endometriosis. Acta Obstet Gynecol Scand 25 377 1946.
22. Wynn, T E.: Endometriosis of the sigmoid colon. Arch Pathol 92. 24, 1971

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Fig 5 Greater enlargement of the m t nant part of Fig. 3 Several layers of epithelium and cellular polymorphism can be seen. x approx. 350

be fulfilled in order that malignant transformation in endometrial cysts can be said to have occurred. Namely:

1 Benign and malignant endometrial tissue should be present in the same ovary

2. The malignant and benign tissue must show the same histological relationship as shown by the endometrial carcinoma in the body of the uterus and the non-malignant part of the uterine mucosa.

3 The carcinoma must originate from the endometrial epithelium and not invade it from without

Scott (20) has later added

4 There must be continuity between the benign and malignant epithelium.

Docherty et al. (6) and Ferreira & Clayton (9) have described two cases each of adenocarcinoma of the endometrial type originating from outside the ovaries, namely from the recto-vaginal septum. However their cases do not comply with the criterion of Scott as far as can be seen from the reports.

In our case all the criteria have been fulfilled and we must therefore presume that the malignancy has originated in a primary endometriosis. Our patient had been sterile for the last ten years and had complained of symptoms which must be attributed to the endometriosis. Ovarian pelvic and intestinal endometriosis had been demonstrated histologically earlier the symptoms of this, which had led to operation, correspond to those previously described (1 11 22) in connection with endometriosis of the sigmoid colon.

Some authors state that granulosa-cell tumours are very often followed by carcinoma of the body of the uterus (5 14). The increased estrogen production is presumed to provoke the development of the carcinoma Others, however have not been able to confirm the increased coincidence of these tumours (2, 7).

If increased estrogen production is presumed to have a carcinogenic effect on the endometrium in the body of the uterus then malignant transformation of ectopic endometrial tissue (endometriotic tissue) is a natural sequence. The simultaneous occurrence of granulosa-cell tumour and malignant transformation of endometriotic tissue has not been described previously but Lauslaht (13) has recently reported a case of simultaneous thecoma together with adenocarcinoma of umbilical endometriosis. Fathella (8) found in two patients with theca-granulosa-cell tumours, post menopausal hyperplasia in the endometriotic cysts in the opposite ovary and Plate (17) has found follicular cysts with hyperthecosis in the ovaries of a patient with clear-cell adenocarcinoma in endometrioid ovarian cysts.

In consideration of the above it is natural to presume that the granulosa-cell tumour in our patient has provoked the rare malignant transformation in the endometriotic tissue. The occurrence of endometrium in the secretory phase is not uncommon in patients with granulosa-cell tumours and does not confuse the suggested relationship.

REFERENCES

1. Aaberg, C.: Studier over endometriosens klinik, p. 210. Munksgaard, Copenhagen, 1944
2. Busby T & Anderson, G. W Feminizing mesenchymomas of the ovary Am J Obstet Gynecol 68 1391, 1954.
3. Clemmesen, J Statistical studies in malignant neoplasms, p. 29 Munksgaard, Copenhagen, 1965.
4. Crist, T Bremer W E. & Edwards, J R.: Adenoacanthoma originating in endometriosis of the ovary Obstet Gynecol 37 419 1971.
5. Dobbie, A. W Granulosa and theca cell ovarian tumors prognosis. Cancer 5 215 1952.
6. Dockerty M. B Pratt, J H & Decker D. G., Primary adenocarcinoma of the rectovaginal septum probably arising from endometriosis. Cancer 7 893, 1954
7. Emge, L. Endometrial cancer and feminizing tumors of the ovaries. Obstet Gynecol 1 511, 1953.
8. Fathalla, M. F Malignant transformation in ovarian endometriosis. J Obstet Gynaecol Br Commw 74 85, 1967
9. Ferreira, H. P & Clayton, S G Three cases of malignant change in endometriosis, including two cases arising in the recto-vaginal septum. J Obstet Gynaecol Br Commw 65 41, 1958.
10. Gray L. A. & Barnes, M. L. Endometrioid carcinoma of the ovary Obstet Gynecol 29 694 1967
11. Jacobsen, B. B & Harting, B. E.. Endometrioid cell sarcoidal. Ugeskr Laeger 134 541 1972.
12. Jenkinson, E. L. & Brown, W H.. Endometriosis. JAMA 122 349 1943
13. Leonhardt, Kalevi. Malignant external endometriosis. Acta Pathol Microbiol Scand, Sect. A, Suppl. 233: 91, 1972.
14. Marcell, H. & Hertig, A. T Granulosa-theca cell tumors. Obstet Gynecol 6 385 1955
15. Masson, J C.. Surgical significance of endometriosis. Ann Surg 102 819 1935
16. Moertel, C. G.. Multiple primary malignant neoplasms, their incidence and significance. Recent Results in Cancer Research, vol. 7 p. 22. Springer Verlag, Berlin, Heidelberg and New York, 1966.
17. Plate, W P Clear-cell adenocarcinoma in an endometrioid ovarian cyst. Obstet Gynecol 27 428 1967
18. Ridley J H Primary adenocarcinoma in implant of endometriosis. Obstet Gynecol 27 261 1966.
19. Sampson, J A.: Endometrial carcinoma in ovary arising in endometrial tissue in that organ. Arch Surg 10: 1 1925
20. Scott, R. B.. Malignant changes in endometriosis. Obstet Gynecol 2. 283 1953.
21. Tellum, G Carcinoma arising in ovarian endometriosis. Acta Obstet Gynecol Scand 25 377 1946.
22. Wynn, T R.. Endometriosis of the sigmoid colon. Arch Pathol 92. 44 1971

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Fig. 5. Greater enlargement of the malignant part of Fig. 3. Several layers of epithelium and cellular polymorphism can be seen. × approx. 380.

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REFERENCES

1. Aaberg, C.: Studier over endometriomernes klinik, p. 210. Munksgaard, Copenhagen, 1944.
2. Busby T. & Anderson, G. W.: Feminizing mesenchymomas of the ovary. Am J Obstet Gynecol 68. 1391, 1954.
3. Clemmesen, J. Statistical studies in malignant neoplasms, p. 29. Munksgaard, Copenhagen, 1965.
4. Crist, T. Bruner W. E. & Edwards, J. R.: Adenoacanthoma originating in endometriosis of the ovary. Obstet Gynecol 37 419, 1971.
5. Diddle, A. W. Granulosa and theca cell ovarian tumors: prognosis. Cancer 5 215 1952.
6. Dockerty M. B. Pratt, J. H. & Decker D. G.: Primary adenocarcinoma of the rectovaginal septum probably arising from endometriosis. Cancer 7 893, 1954.
7. Emge, L.: Endometrial cancer and feminizing tumors of the ovaries. Obstet Gynecol 1 511 1953.
8. Fathalla, M. F. Malignant transformation in ovarian endometriosis. J Obstet Gynaecol Br Commw 74 85, 1967.
9. Ferreira, H. P. & Clayton, S. G.: Three cases of malignant change in endometriosis, including two cases arising in the recto-vaginal septum. J Obstet Gynaecol Br Commw 65. 41, 1958.
10. Gray L. A. & Barnes, M. L. Endometrioid carcinoma of the ovary. Obstet Gynecol 29 694 1967.
11. Jacobsen, B. B. & Hartling, S. E.: Endometriosis coli sigmoidei. Ugeskr Laeger 134 541, 1972.
12. Jenkinson, E. L. & Brown, W. H.: Endometriosis. JAMA 122 349 1943.
13. Lauslahti, Kalevi: Malignant external endometriosis. Acta Pathol Microbiol Scand, Sect. A, Suppl. 233 98, 1972.
14. Mansell, H. & Hertig, A. T. Granulosa-theca cell tumors. Obstet Gynecol 6 385 1955.
15. Masson, J. C.: Surgical significance of endometriosis. Ann Surg 102 819 1935.
16. Moertel, C. G. Multiple primary malignant neoplasms, their incidence and significance. Recent Results in Cancer Research, vol. 7 p. 22. Springer Verlag, Berlin, Heidelberg and New York, 1966.
17. Plate, W. P. Clear-cell adenocarcinoma in an endometrioid ovarian cyst. Obstet Gynecol 27 428 1967.
18. Ridley J. H. Primary adenocarcinoma in implant of endometriosis. Obstet Gynecol 27 261 1966.
19. Sampson, J. A.: Endometrial carcinoma in ovary arising in endometrial tissue in that organ. Arch Surg 10 1 1925.
20. Scott, R. B. Malignant changes in endometriosis. Obstet Gynecol 2 283 1953.
21. Teilum, G.: Carcinoma arising in ovarian endometrioses. Acta Obstet Gynecol Scand 25 377 1946.
22. Wynn, T. E.: Endometriosis of the sigmoid colon. Arch Pathol 92. 24 1971.

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THE EFFECT OF OXYTOCIN ON LOCAL UTERINE BLOOD FLOW IN WOMEN WITH SECONDARY AMENORRHOEA AND EARLY PREGNANCY MEASURED BY LOCAL HYDROGEN CLEARANCE

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Abstract. Local uterine blood flow was measured by the hydrogen gas clearance method in 36 women before and after injection of oxytocin. In women with secondary amenorrhoea mean myometrial blood flow was 99.0 ± 1.75 ml/min 100 g. No or only slight reduction of myometrial blood flow was obtained after administration of oxytocin. During the first few days after therapeutic abortion performed in the first trimester of pregnancy mean myometrial blood flow was 124.3 ± 15.71 ml/min 100 g. After injection of oxytocin, myometrial blood flow was reduced to mean of 19.7 ± 6.07% of controls. In the first trimester of pregnancy and during the first few days after therapeutic abortion, cervical uterine blood flow was not or only slightly reduced after administration of oxytocin.

In a previous study (8) myometrial blood flow in women of fertile age was found to be significantly higher than in postmenopausal women. Furthermore, myometrial blood flow was considerably reduced during the first few minutes after an injection of oxytocin in women of fertile age, while this effect was apparently lacking in postmenopausal women. The differences in uterine blood flow pattern of women of fertile age and of postmenopausal women might be due to the hormonal milieu.

To obtain further information about the influence of endocrine factors on local uterine blood flow groups of patients with hormonal differences were selected. Thus myometrial blood flow was measured before and after injection of oxytocin in women with secondary amenorrhoea and in women after legal abortion. In addition the influence of oxytocin on cervical blood flow was studied in the first trimester of pregnancy and in some cases also after delivery.

METHODS

Uterine blood flow measurements were performed by the local hydrogen gas clearance method (2). The platinum electrodes were inserted into the fibro-muscular tissue of the cervix (5) and through the cervical canal into the myometrium of the fundus uteri (6) without anaesthesia. The intravenous administration of a single dose of oxytocin, 5 IU (Pitocin, Parke, Davis & Company) and the estimation of blood flow was made as described previously (8).

Endometrial biopsies were taken within 24 hours after blood flow measurements, and the patients were classified according to the result of the microscopical examination (9). Before the blood flow measurements urinary oestriol analyses (1) were performed in 48 hour urine specimens.

Differences of blood flow between groups were tested by the Wilcoxon rank test and the Student's *t*-test. In the tables (I, II and III) mean flow of the groups ± S.E. were calculated from the mean value in each patient.

MATERIAL

Uterine blood flow measurements before and after injection of oxytocin were made in 36 patients divided in 4 groups:

1. Secondary amenorrhoea: 13 patients
2. Pregnancy 1st trimester, before legal abortion: 9 patients
3. Pregnancy 1st trimester, after legal abortion: 10 patients
4. Pregnancy at term, after delivery: 4 patients

The patients with secondary amenorrhoea (*Table I*) were between 20 and 31 years old, and their amenorrhoea had lasted from 5 to 120 months. The menarche had occurred between 11 and 17 years of age, and most of the patients had previously had regular menstrual periods. Obvious clinical features of premature menopause could not be observed among these patients. Three of them (Nos. 9, 11 and 12) had previously had children. The cervical canal and uterine cavity measured together 6 cm or more in all patients except one.

Table I *Effect of oxytocin on myometrial blood flow in secondary amenorrhoea*

							Myometrial blood flow					
							Before oxytocin		After oxytocin			
							Ml/min 100 g		Ml/min 100 g		In percent o the flow bef oxytocin	
Pat. no.	Histology of the endometrium	Age (y)	Menarche	Previous menstrual type	Amenorrhoea (months)	Urinary oestriol (µg/24 h)	Each electrode	Mean	Each electrode	Mean	Each electrode	Me
1	Atrophic	30	13	Regular	120	7.5	86.6 109.5	98 1	69.3 74.3	71.8	80 0 67.9	74[illegible]
2	Atrophic	31	13	Regular	48	10.9	138.6 165 0	151.8	138 6 119.5	129 1	100.0 72 4	8[illegible]
3	Atrophic	23	14	Regular	26	5 4	110.0 86.6	98.3	30 1 [illegible]3 1	26.6	27 4 [illegible]6 7	27.[illegible]
4	Atrophic	[illegible]3	13	Regular	23	7 6	146.3 122.2	134.3	1[illegible]2.2 109.5	115 9	83.5 89 6	86[illegible]
5	Atrophic	20	13	Regular with prolonged intervals	[illegible]0	9 6	157 0 188.8	172.9	106 6 1[illegible]2	114 4	67 9 64 7	66.3
6	Atrophic	[illegible]3	14	Regular	15	18 1	133 0	133 0	115.5	115.5	86 8	86 1
7	Atrophic	22	13	Regular	11	7.2	86 6 81.5	84 1	71 1 69.3	70 2	82.1 85 0	83 6
8	Atrophic	21	17	Regular	9	5 8	43 4 54.3	48.9	36.5 30 9	33.7	84.1 56 9	70.5
9	Inactive	23	11	Regular	5	10.8	99 0 92.4	95 7	14 0 [illegible]3 1	18 6	14.1 [illegible]5 0	19 6
10	Proliferative phase with regressive changes	23	15	Regular	36	18.6	49.5 36.5	43 0	42 0 30.1	36.1	84 8 82.5	83 7
11	Proliferative phase with regressive changes	27	14	Regular	23	13.9	36.5	16.5	23.9	[illegible]3 9	63.5	65 5
12	Proliferative phase with regressive changes	24	14	Regular with prolonged intervals	6	17 8	144 5	144 5	54 6	54.6	37.8	37 8
13	Proliferative phase with slight cystic glandular hyperplasia	6	16	Irregular with prolonged intervals	7	8.2	46 0	46 0	15 1	15 1	3[illegible] 8	3[illegible]8
Mean ± S E.								99 0 ±12 75		61 5 ±11 73		63 1 ±6 9[illegible]

Patients admitted for legal abortion were investigated during different conditions. In the group studied before termination of pregnancy (Table II) blood flow was only recorded in the cervical tissue In the other group (Table III) both myometrial and cervical blood flow was measured, in five of the patients on the second day fter termination of pregnancy and on the f fth to eight day in the other f The patients in the two grou w re not identical.

The cervical blood flow was measured after nevent pregnancies and deliveries at term (Table IV) in th last group.

Table II. *Effect of oxytocin on uterine cervical blood flow in 1st trimester of pregnancy*

			Blood flow before oxytocin		Blood flow after oxytocin			
			Ml/min 100 g		Ml/min 100 g		In percent of flow before oxytocin	
Pat. no.	Age (y.)	Weeks of pregnancy	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean
28	24	12	57.8 80	69.0	49.5 69.3	59.4	85.6 86.4	86.0
29	33	12	60.2 40.8	50.5	40.8 26.7	33.8	67.8 65.4	66.6
30	31	10	99.0 132.0	115.5	73.0 92.4	82.7	73.7 70.0	71.9
31	27	12	77.0 66.0	71.5	77.0 55.4	66.2	100.0 83.9	92.0
32	33	10	138.6 154.0	146.3	77.0 92.4	84.7	55.6 60.0	57.8
33	45	10	86.6 86.6	86.6	86.6 99.0	92.8	100.0 114.3	107.2
34	26	10	94.9 115.0	105.0	123.8 134.3	129.1	130.5 116.8	123.7
35	23	12	74.3	74.3	53.3	53.3	71.7	71.7
36	27	11	80.0	80.0	57.8	57.8	72.3	72.3
Mean SE				88.7 ±9.66		73.3 ±9.24		83.2 ±7.07

RESULTS

The blood flow measurements from 22 different areas in the myometrium in the patients with secondary amenorrhoea (Table I) showed a mean flow on 99.0 ± 12.75 ml/min 100 g. Two minutes after injection of oxytocin mean flow was reduced to 63.1 ± 6.90% of controls.

In Table I the patients are listed according to the endometrial picture of the endometrium. In the 8 patients with atrophic endometrium blood flow fell to mean of 72.6% of controls. Three patients with five, six and seven months of amenorrhoea respectively had a mean flow reduction to 30.1% of controls. Nine out of ten patients with a duration of amenorrhoea of nine months or more showed a mean flow decrease to 78.1% of flow before administration of oxytocin. Patient 3 had amenorrhoea for twenty-six months and still the flow as reduced to 27.1% of controls. All patients had urinary oestriol excretion lower lower than 20 μg/24 h.

Nine pregnant women in the first trimester (Table II) had mean cervical blood flow of 88 ± 9.66 ml mm 100 g from 16 different areas. After administration of oxytocin the flow was 83.2 ± 7.07 of controls.

From 18 different myometrial areas in 10 patients who had had a therapeutic abortion performed (Table III), mean blood flow was 124.3 ± 15.71 ml/min 100 g. and a mean flow reduction to 19.7 ± 6.07% of controls was obtained after administration of oxytocin. Measurements on the second day after termination of pregnancy showed a mean flow reduction to 5.7% of controls, while mean flow five to eight days after operation was reduced to 33.7% of controls. Simultaneous measurements of cervical blood flow from 18 areas in 8 of the patients showed mean flow as low as 47.5 ml/min 100 g, reduced to 72.4 ± 4.41% of controls after injection of oxytocin. The time which had elapsed since the termination of pregnancy did not seem to influence this result.

Cervical blood flow measurements in 4 women post partum (Table IV) showed a mean blood flow of 55.6 ml/min 100 g, reduced to 87.7% of controls after injection of oxytocin.

DISCUSSION

The term secondary amenorrhoea is most frequently applied t women under 40 years of age,

Table 1 *Effect of oxytocin on myometrial blood flow in secondary amenorrhoea*

Pat. no.	Histology of the endometrium	Age (y)	Menarche	Previous menstrual type	Amenorrhoea (months)	Urinary oestriol (μg/24 h)	Myometrial blood flow: Before oxytocin, Ml/min 100 g, Each electrode	Before oxytocin, Ml/min 100 g, Mean	After oxytocin, Ml/min 100 g, Each electrode	After oxytocin, Ml/min 100 g, Mean	After oxytocin, In percent of the flow before oxytocin, Each electrode	In percent of the flow before oxytocin, Mean
1	Atrophic	30	13	Regular	1?0	7.5	86.6 109.5	98.1	69.3 74.3	71.8	80.0 67.9	[illegible]
2	Atrophic	31	13	Regular	48	10.9	138.6 165.0	151.8	138.6 119.5	[illegible]	100.0 77.4	[illegible]
3	Atrophic	23	14	Regular	26	5.4	110.0 86.6	98.3	30.1 23.1	26.6	27.4 [illegible]	[illegible]
4	Atrophic	23	13	Regular	23	7.6	146.3 [illegible]	134.3	[illegible] 109.5	115.9	83.5 89.6	[illegible]
5	Atrophic	20	13	Regular with prolonged intervals	?0	9.6	157.0 188.8	172.9	106.6 [illegible]	114.4	67.9 64.7	[illegible]
6	Atrophic	[illegible]	14	Regular	15	18.1	133.0	133.0	115.5	115.5	86.8	86.8
7	Atrophic	[illegible]	13	Regular	11	7.2	86.6 81.5	84.1	71.1 69.3	[illegible]	[illegible] 85.0	[illegible]
8	Atrophic	21	17	Regula	9	5.8	43.4 54.3	48.9	36.5 30.9	33.7	84.1 56.9	[illegible]
9	Inactiv	[illegible]	11	Regular	5	10.8	99.0 [illegible]	95.7	14.0 3.1	18.6	14.1 23.0	[illegible]
10	Proliferative phase with regressive changes	3	15	Regular	36	18.6	49.5 36.5	43.0	42.0 30.1	36.1	84.8 [illegible]	81.7
11	Proliferative phase with regressive changes	7	14	Regular	[illegible]	13.9	36.5	36.5	21.9	[illegible]	63.5	63.5
12	Proliferative phase with regressive changes	4	14	Regular with prolonged intervals	6	17.8	144.5	144.5	54.6	54.6	37.8	37.8
13	Proliferative phase with slight cystic glandular hyperplasia	?6	16	Irregular with prolonged intervals	7	8.2	46.0	46.0	15.1	15.1	[illegible]	[illegible]
Mean ±S.E.								99.0 ±[illegible]		63.5 11.73		63.1 ±6.90

Patients admitted for legal abortion were investigated during different conditions. In the group studied before termination of pregnancy (Table II) blood flow was only recorded in the cervical tissue. In the other group (Table III) both myometrial and cervical blood flow was measured, in five of the patients on the second day after termination of pregnancy and on the fifth to eighth day in the other f[illegible] The patients in the two groups were not identical.

Th[illegible] cervical blood flow was measured after uneventful pregnancies and [illegible] term (Table IV) in the last group.

had about the same mean local myometrial blood flow as women with intact ovarian function, but compared with postmenopausal women, the flow rate was considerably higher (8). It could therefore be expected that patients with prolonged amenorrhoea might also show a reduced uterine blood flow. However no correlation between local blood flow rate and the duration of amenorrhoea was observed, although the variation from one patient to another could be large in some cases.

Women of fertile age with intact ovarian function showed myometrial blood flow reduction to 20–30% of controls after administration of oxytocin, while no effect could be observed in post menopausal women (8). In most of the women with secondary amenorrhoea there was no, or only a slight, effect of oxytocin on myometrial blood flow though some more pronounced reduction was obtained when the amenorrhoea had been of short duration. If the reduction of myometrial flow after injection of oxytocin was due to external compression of vessels, the absent or slight reduction in women with secondary amenorrhoea might indicate a decreased myometrial response on oxytocin or a smaller amount of muscle fibres in the myometrium as described in postmenopausal women (10). Women with secondary amenorrhoea were similar to women of fertile age as to the magnitude of local myometrial blood flow but similar to postmenopausal women as to the effect of oxytocin. Therefore the lowered endocrine function in the ovaries in women with secondary amenorrhoea seemed to influence the myometrial blood flow pattern, either directly or by inducing secondary changes in the myometrial tissue.

Mean myometrial blood flow rate during the first few days after therapeutic abortion was higher than in non-pregnant women of fertile age (8). This is in agreement with the observation of Jansson (4), who found the highest myometrial Xe^{133} clearance rate in the puerperium. The marked reduction of myometrial blood flow after injection of oxytocin on the second day after termination of pregnancy was probably due to uterine contractions, since the patients all felt pains in the lower abdomen. The definite increase in the amount of muscle fibres during pregnancy observed by Schwalm and Dubrauszky (10) supports this assumption. Both the marked effect of oxytocin on myometrial blood flow and the slight or absent reduction of cervical blood flow after termination of pregnancy is in accordance with previous observations in non-pregnant women of fertile age (8). The observation that the influence of oxytocin seemed to decrease during the post abortion period corresponds with the results obtained in sheep by Assali et al. (1) using an electromagnetic flowmeter applied on the uterine artery.

While the myometrial blood flow was high during the post-abortion period, the cervical blood flow seemed not to be raised, and was of the same size as post-partum. Compared with cervical flow post-abortion and post-partum, cervical flow during the first trimester of pregnancy seemed to be higher. Cervical flow in women with regular menstrual periods (8), in women of fertile age with metrorrhagia (5) and in women with carcinoma of the cervix uteri (7) was of similar magnitude as in the first trimester of pregnancy. As obtained in women with regular menstrual periods (8) no or only a slight, reduction of cervical blood flow seemed to occur after injection of oxytocin in the first trimester of pregnancy. The small number of muscle fibres in the uterine cervix and the small increase in cervical muscle fibres during pregnancy (10) suggest that external compression of vessels by the contracting myometrium might be the main reason for the effect of oxytocin on uterine blood flow.

ACKNOWLEDGEMENT

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REFERENCES

1. Assali, N. S., Dasgupta, K. & Kolin, A. Measurement of uterine blood flow and uterine metabolism. Amer J Obstet Gynecol 78 313–321, 1959
2. Aukland, K., Bower B. F & Berliner R. W. Measurement of local blood flow with hydrogen gas. Circ Res 14 164–187 1964.
3. Eberlein, W. R., Bongiovanni, A. M. & Francis, Catherine M.: A simplified method for the routine measurement of urinary estriol. J Clin Endocrinol 18, 1274–1284 1958.
4. Jansson, L. 133Xenon clearance in the myometrium of pregnant and non-pregnant women. Acta Obstet Gynecol Scand 48 302–321 1969
5. Klingenberg, I & Aukland, K. Measurement of human uterine cervical blood flow by local hydrogen gas clearance. Acta Obstet Gynecol Scand 48 455–469 1969

Table III *Effect of oxytocin on uterine blood flow after therapeutic abortion*

				Myometrial blood flow						Cervical blood flow					
				Before oxytocin		Two minutes after oxytocin				Before oxytocin		After oxytocin			
			Days after thera-peutic abor tion	Ml/min 100 g		Ml/min 100 g		In percent of the flow be-fore oxytocin		Ml/min 100 g		Ml/min 100 g		In percent of the flow before oxytocin	
Pat. no	Age (y)	Weeks of preg-nancy		Each elec trode	Mean	Each elec trode	Mean	Each elec trode	Mean	Each elec trode	Mean	Each elec trode	Mean	Each elec trode	Mean
14	28	13	2	173.3 225 0	199.2	3 9 2.8	3 4	2.3 1.2	1.8	38.5 30 1 30.8	33 1	36.5 28.3 26.7	30.5	94 8 94 0 86.7	91 8
15	33	8	2	84 0 106.6	95.3	8 7 12.8	10.8	10.4 12.0	11.2	60.3 27 7 74.9	54.3	39.6 15.9 53.3	36.3	65.7 57 4 71.2	64 8
16	27	11	2	59 0 50.4	56.7	6.5 0.1	3.3	11.0 0.2	5 6	33 0 28.0	30.5	26.2 25 4	25.8	79.4 90.7	85 1
17	22	13	2	92.4 79 9	86.2	4 7 8.5	6.6	5 1 10.6	7.9	71 7 70.5	71 1	43.3 40.8	42.1	60.4 57 9	59.2
18	29	13	2	148 4	148 4	3.3	3.3	2.2	2.2						
19	24	7	5	81.5 120.5	101.0	46.2 34.7	40.5	56.7 28.8	42.8	40.8 55.4	48.1	24.8 42.0	33 4	60.8 73.8	67 3
20	41	10	5	208 1 208 1	208.1	99 0 49 5	74.3	47 6 23 8	35 7	77 0 43.3 63.0	61 1	46.2 19 8 45.3	37 1	60 0 45.7 71.9	59.2
21	41	12	5	86.6 89 4	88.0	34 7 23.9	29.3	40.1 26.7	33 4	47.8	47.8	39 6	39 6	82.8	82 8
22	39	8	6	184 8 94.5	139 7	3 7 6.0	4.9	2.0 6.3	4.2	42.0 25.2	33 6	23 1 20 4	21 8	55 0 81.0	68 0
23	37	12	8	120.5	120 5	63 0	63 0	52.3	52.3						
Mean ±S.E.					124.3 ±15 71		23.9 ±8.48		19 7 ±6.07		47.5 ±5.15		33.3 ±2.45		72 4 ±4 41

who have had amenorrhoea for at least 4 months. They might previously either have had regular menstruation for some years, prolonged intervals, or only bled scantily. The group comprising 13 women in the present investigation agreed with this definition. Usually the menstrual disorders reflect some form of ovarian failure. In the present study this was supported by the fact that all patients had abnormal endometrium and a low excretion of urinary oestriol. These women

Table IV *Effect of oxytocin on uterine cervical blood flow post partum*

			Before oxytocin		After oxytocin			
			Ml/min 100 g		Ml/min 100 g		In percent of flow before oxytocin	
Pat. no	Age (y)	Days post partum	Each electrode	Mean	Each electrode	Mean	Each electrode	Mean
24	32	9	41.0	41.0	30.0	30.0	71 2	73 2
25	38	6	60.0 44 0	52.0	53.0 44 0	49.5	91 7 100 0	95 9
26	29	11	77 0 74.9	76.0	79.2 86.6	82.9	102.9 115 6	109 1
27	34	12	53.3	53.3	38.5	38 5	72.2	72.2
Mean				55 6		50.2		87 7

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ENDOCRINE CHANGES BEFORE AND AFTER THE MENARCHE

I. Urinary Excretion of Estrogen, FSH and LH and Serum Levels of Progesterone FSH and LH

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Abstract. Serum and urine samples from 148 healthy female children and adults were studied, ranging from girls with a bone age of 8 years to adulthood. All samples from menstruating girls were obtained between the 7th and 10th days of the menstrual cycle. The blood sample was taken on the same day as the collection of 24-hour urine sample was completed. The purpose of the study was to investigate the endocrine processes, with as many parameters as possible, before and after the menarche. In assessing the results account was taken of the bone age, the gynecological age and Tanner's puberty classification into 5 groups. Our results show that girls' sexual and endocrine development had begun before they reached a bone age of 8 years. In the present series the FSH level at the age of 8 equalled the low adult follicular phase level, 1.12 ng/ml, and by the menarche it had increased to 1.84 ng/ml. In the lowest age group of the present series, 8 years, the mean LH level was 0.70 ng/ml, and increased until at the menarche it was 1.19 ng/ml. The initial value for total urinary estrogens at the age of 8 was 4.63 µg/24 h and increased until at the menarche it was 10.20 µg/24 h. From this level the estrogen excretion continued to increase for another 3 years, until at the age of about 16 years it reached an adult level of about 17 µg/24 h. According to this study the menarche seems to be followed by a steady phase of some 18–24 months in hormone excretion, with practically all hormone values remaining constant. Hormone excretion in girls around the age of puberty seems to show a slow, almost linear increase, without any remarkable peaks before the menarche, and the endocrine maturation seems to continue up till 3 years after the menarche.

According to current opinion, the development of a girl into a sexually mature woman is essentially governed by the estrogen hormones.

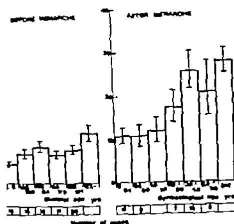
Estrogen of maternal and placental origin can be shown in the neonate during the first 5–6 days of life (11). Although at the moment of birth there are several hundreds of thousands of primordial follicles in the ovary, the estrogen secretion of the ovaries during the first year of life is below or on the borderline of detection by the methods used (11).

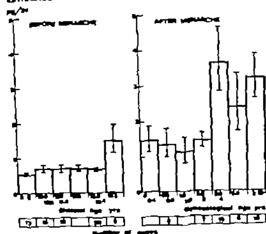
During infancy and childhood the primordial follicles may undergo varying degrees of maturation to the stage of the early graafian follicle. However, these follicles do not secrete larger amounts of estrogen until the hypophysarian-ovarian feedback system has matured. During the latter part of adolescence, LH is elaborated and acts, probably synergistically with FSH, to cause more estrogen production. The first meiotic division of the ovum occurs under the stimulus of LH. However, at this age, not all estrogen is of ovarian origin, some is of adrenal origin. According to most authors, the estrogen secretion in girls begins to increase around the 8th year of life (15, 28, 40). Disagreement prevails in the literature concerning the changes in the secretion of estrogens during puberty, especially about the time of menarche. The most quoted paper is that by Nathanson et al. (28). Using a biological method, the authors showed that, in girls, estrogen secretion increased sharply, by 6 times, after the age of 8 to 13 years. No corresponding increase was recorded in boys. By a study based on a new chemical method (7), Pennington & Dewhurst (31) showed that it is nearly always pos-

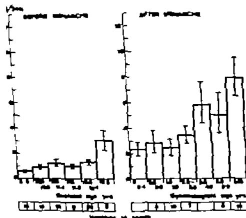
6. Klingenberg, I Measurement of blood flow in human myometrium by local hydrogen clearance Acta Obstet Gynecol Scand *48* 470–478, 1969
7 — The effect of radium on blood flow in the human uterine cervix measured by local hydrogen clearance Acta Obstet Gynecol Scand *53* 7 1974
8 — The effect of oxytocin on human uterine blood flow measured by local hydrogen clearance Obstet Gynecol Scand *53*. 103 1974
9 Myhre E. Endometrial biopsy and ovarian hormonal failure Acta Obstet Gynecol Scand 45 Suppl. 9 143–157 1966.
10. Schwalm, H, & Dubrauszky V The structure of the musculature of the human uterus—muscle and connective tissue. Amer J Obstet Gynecol 94 391–[illegible] 1966

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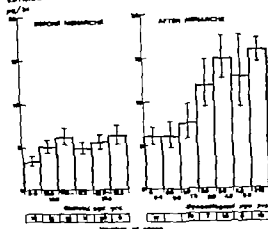


Fig. 1. The excretion of total estrogens, estradiol, estrone and estriol in pre- and postmenarcheal girls.

of menarche of all the menstruating girls was 12.2 years (S.D. 1.6 years). The skeletal age of the girls who had menstruated less than 6 months was 12.8 years and the chronological age 12.9 years.

The purpose of the study was to group the girls, as precisely as possible, according to their biological maturity. For this reason, the bone age of the premenarcheal girls was used, since it correlates better with the menarche than the chronological age (25). The grouping of the menstruating girls was based on the so-called gynecological age which refers to the time that has passed since the menarche (30). In the later presentation of the results, the date of the menarche is indicated by a break in the curves. On the basis of the foregoing, the date of the menarche seems to occur at approximately the 13th year of bone age, and therefore the relevant curves presumably can be combined.

The 24-hour urine was collected by the girls at home according to meticulous instructions. The girls whose menstrual periods were even approximately regular collected it within the 7th to 10th days of the beginning of the preceding period. The preserving agent used was mixture of chloroform and ethanol.

A venous blood sample was taken in the afternoon after the collection of the 24 h urine specimen was finished.

The determinations of *estrone*, *estradiol* and *estriol* from the urine were carried out using the method described by Adlercreutz et al. (1, 2). This method is based on the methods by Brown (7) and Beling (3), and includes gel filtration of the urinary conjugated estrogens, enzymatic hydrolysis of the conjugates, solvent partition, methylation and chromatography of the methylated estrogens. The same technique has been used in the study

sible to detect estrogens in the urine of young girls, at least from the 3rd year of age onwards. In premenarcheal girls the graph of the normal range showed an upward trend with increasing age and secondary sexual development. The increase of estrogen in the series of Pennington & Dewhurst (31) was very moderate up to the age of 14 and the findings disagreed with those of Nathanson et al. (28). Neither was Jayle (16) able to confirm such a high premenarcheal increase of estrogens. He pointed out that even after the menarche it takes a long time before the relatively low estrone estradiol and estriol values reach a level equal to the cyclic secretion of the fertile woman (13)

Data given in the literature concerning the changes in estrogen secretion immediately after the menarche is extremely limited. Using a highly sensitive radioimmunoassay technique applicable to the measurement of 17-beta-estradiol in plasma Jenner et al. (17) studied a group of pre- and postmenarcheal girls. They found that the plasma E_2 rose slowly but steadily with advancing sexual maturation and correlated especially well with the clinical evaluation of pubertal development It is apparent that, by means of very sensitive hormone determination estrogens are demonstrable in the plasma of even very young girls, and that the rise of estrogens in puberty is slow and steady without any remarkable peaks before the onset of menarche.

The role of gonadotrophins before the menarche has been unclear in many respects. Earlier authors, using less sensitive methods, were often unable to show measurable amounts of LH or FSH in younger children (8 12, 22 26 27 30 31 34 37). Yen Vicic & Kearchner (51) found detectable amounts of LH from the 8th year on in both boys and girls. The LH secretion was progressive up to sexual maturity and the increase of LH was 6-fold in girls. Lee et al (24) found an earlier rise of both LH and FSH in girls than boys, whereas the rise in gonadotrophins in their study failed to correlate with the pubertal development and the initial appearance of the physical characteristics of puberty Saxena et al. (36) Penny et al. (32) Blizzard et al (5) Sizonenko et al. (38 39) found a significant increase in serum FSH in 5 to 8-year-old prepubertal girls, while the LH rise was not significant until the age of 9–10 years. Jenner et al. (17) showed that the first hormonal event in female puberty is an increased secretion of gonadotrophins, especially FSH. Hayes & Johanson (14) reported that the excretion of LH and FSH in premenarcheal girls followed no constant pattern, but the average levels were comparable with postmenarcheal and adult levels for FSH and early postmenarcheal levels for LH.

On the basis of recent studies it can be concluded that the hypothalamo-pituitary-gonadal axis is operative already in the prepubertal child and that the sensitivity of the gonadostat to exogenous sex steroids decreases at the onset of puberty (17)

Progesterone is mainly produced in the ovary but certain observations suggest that there is a small constant production of progesterone in the adrenals of both sexes (21). Bergstrand & Gemzell (4) demonstrated that pregnanediol excretion in children did not differ from that found by Klopper et al (21) in adult men and postmenopausal women.

The present study was cross-sectional. The girls were examined during the same cyclical phase for 24-hour excretion of all the hormones mentioned, and the results during the various phases of puberty were reviewed (48).

MATERIAL AND METHODS

The series consisted of 148 schoolgirls and training nurses. Their ages ranged from 7 to 20 years. Premenarcheal girls numbered 80 and menstruating girls 68

The study was made as a cross-sectional investigation of the series The clinical examination of all the subjects was carried out by one of the authors (R L K). All the girls were healthy as judged by their medical histories and clinical examinations. Heights ranged from 122.3 to 175 0 cm and weights from 1 3 to 72 0 kg When the means were plotted onto the Finnish standard curve (9), it was seen that both the height and weight means fell very close to the 90th percentile curve

A hand X-ray was taken of all the girls for the determination of bone age using the Tanner Whitehouse method (41), which has been found well-suited to Finnish children (42, 43) All determinations were carried out by one of the authors (R L K), who was familiar with the method The bone ages of the series ranged from 8.0 years to adulthood.

The stage of puberty was determined by clinical examination according to breast and pubic hair ratings, as described by Tanner (40). In the girls whose menarche had taken place less than 6 months previously the mean for the breast stage was 3 44 (S.D 0.5) and the mean pubic hair stage 3.39 (S.D 0 6). The mean age

Table II. *Urinary excretion of total estrogens according to skeletal age µg/24 h*

Skeletal age, y	N	Mean	M− S.E.M.	M+ S.E.M.
8–9.9	13	4.63	4.10	5.22
10–10.9	15	7.02	6.09	8.10
11–11.9	26	7.30	6.57	8.07
12–12.9	37	10.20	9.39	11.26
13–13.9	15	9.81	8.68	11.10
14–14.9	17	14.70	11.95	18.00
15–adult	17	20.80	17.43	24.85

a steeper increase about 18 months after the menarche. The estradiol and estrone columns show sharp increase in the mean values when the bone age advances from 12 to 13 years, apparently just before the menarche. This point of time marks the level at which the excretion remains for about 2 years after the menarche. Subsequently the excretion of all three estrogen fractions is doubled from the menarche to the 5th gynecological year. The pre- and postmenarcheal mean values show a highly significant difference ($p<0.001$) both for total estrogen and its fractions.

The estrogen fraction ratio, estradiol/estrone/estriol, obtained for premenarcheal girls was at 8–10 years 1 : 1 : 7 and at 12–14 years 1 : 2 : 7. For menstruating girls it was 1 : 2 : 5 in the first four gynecological years and 1 : 2.4 : 5.7 later.

Table II shows the mean excretion of total estrogen distributed according to the bone age,

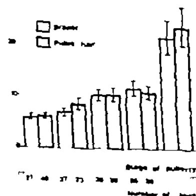


Fig. 2. Total estrogen excretion according to stages of Puberty.

Table III. *Estrogen excretion at the beginning (I) and at the end (II) of menstrual cycle. 14 girls. µg/24 h*

	Mean	Mean − S.D.	Mean + S.D.
Estrone			
I	2.23	1.47	3.35
II	5.95	3.13	11.30
Estradiol			
I	1.02	0.63	1.64
II	2.59	1.47	4.56
Estriol			
I	6.64	4.39	10.00
II	13.20	6.82	26.10
Total estrogens			
I	10.40	7.74	13.86
II	23.41	9.55	42.31

i.e. with menstruating and premenarcheal girls combined. The excretion shows an almost linear rise and the standard errors of the mean are very great after the skeletal age of 13 years. It is seen that the total estrogen excretion from the 9th to 17th years of bone age increased about four fold.

Fig. 2 illustrates the total estrogen excretion according to both pubic hair and breast development ratings. Viewed from this angle, a very steep increase in excretion is visible from stage 4 to stage 5. The patterns are almost identical. In other words, breasts and pubic hair develop in parallel with increasing estrogen excretion. The total estrogens correlate best with skeletal age (r 0.45, $p<0.001$), with breast stage (0.43, $p<0.001$) and with pubic hair stage (0.4.., $p<0.001$). Correlation coefficients to height and weight were slightly lower, yet highly significant.

To obtain an idea of the cyclic estrogen excretion in the series, the 24-hour excretion of 14 young women who had menstruated for not less than 5 years was recorded in the early follicular and the luteal phase, i.e. at the 3rd–7th and 20th–22nd days of the cycle (Table 3). The excretion of all fractions in the latter half of the cycle was slightly more than twice that of the first half; the differences were highly significant for total estrogen and estrone ($p<0.001$) and significant for estradiol and estriol ($p<0.01$).

Progesterone

The distribution of the serum levels of progesterone can be seen from Fig. 3. In the premen-

Table I *Urinary excretion of total estrogens, estrone estradiol and estriol in pre- and post-menarcheal girls, μg/24 h*

	Total estrogens			Estrone		Estradiol		Estriol	
	N	Mean	M∓S.E.M	Mean	M∓S.E.M	Mean	M∓S.E.M	Mean	M S.E.M
Before menarche									
Skeletal age y									
8.0–9 9	13	4.63	4.10–5.22	0.60	0.54–0 67	0.54	0.49–0.60	3.32	2 84–3.8
10 0–10.9	15	7 02	6 09–8 09	0.92	0.74–1 14	0.69	0.61–0 78	5 00	4 33–5.7
11 0–11.5	12	8 46	7 29–9.82	1.23	0 98 1 53	0.70	0 61–0 79	6.11	5 4–7.11
11 6–11 9	11	6.67	5 90–7.55	1 00	0 84–1 70	0.71	0.63–0.80	4.77	4 17–5 8
12.0–1_.1	20	7 82	6.88–8.90	1.29	1 10–1.52	0 67	0.63–0 72	5 48	4 74–6 4
12.5–13 8	9	11 59	9 94–13 50	2.97	_.27–3 86	1 48	1 14–1 93	6 22	3.24–3.9
Total	80	7 28	2.41 22.00	1 12	1 03–1.23	0 72	0 68–0.76	5 0	4 71–5 8
After menarche									
Gynecological age y									
0.0–0 5	17	10 72	9 09–12.65	2.29	1 83–2.87	1.46	1 15–1.86	6 01	5.1 7.8
0.9	8	10 65	8 46–13 42	2.80	2.08–3 80	1 34	1 01 1 79	6.01	4 72–7.6
1 0–1 9	10	11 91	9 37 15 13	2.39	1 82 3 12	1 12	0.82 1 15	7 48	5 78–9 7
2.0–_.9	7	17 14	13 93–21 08	3 37	2.76–4 13	1 45	1.26–1 68	11 76	9 40–14 .4
3 0–3 9	10	25 48	1.57 30 10	5 76	4 38–7 57	3.62	_.83–4 65	14 82	1_.60–17.8
4 0–9 9	6	20 7	15 37–27 90	4 94	3 63–6.73	2.37	1 70–3.31	1_.75	9 27–17.5
> 10	10	27 80	25 10–30.80	7 83	6.51 9 42	3 70	_.64–3 88	15 77	14 47 17.3
Total	68	15.81	14 50–17 24	3 60	3.23–4 02	1 87	1 67 _.10	9 35	8.56–10.2

by Procopé (33) concerning the estrogens in postmenopausal women.

Progesterone in serum was measured according to the method of Neill et al (29) One ml of serum was e tracted once with 10 ml of petroleum ether The xtract was further purified by thin-layer chromatography utiliz ing prewashed silica gel sheets Th influence of the silica gel eluate on the competitive protein binding system was diminished by adding luent to th standard curv (19) The detection limit in this system was 0 2 ng/ml. The values were corrected for procedur l losses Du to lack of serum only sl gl samples wer processed

Immunoreactive FSH and LH in serum and urine wer assayed by a radioimmunosorbent technique (45 46) *FSH in serum and urine* was measured by utilizing human pituitary FSH (35) labelled with ^{125}I and guinea pig a ti-human pituitary FSH. The FSH preparation had biological acti ity of 1 14 000 IU (2nd IRP HMG) per mg. The results of the assay of FSH in serum were expressed i ng/ml using the purified FSH preparation as a provisional standard. One ng of FSH a equivalent to 369 ng of LER-907 in the immunoassay Result from assay of FSH in urine were pressed in IU of the 2nd IRP for HMG per 4 hours.

LH in serum was measured by using huma pituita LH (35) labelled with ^{125}I and abbit nti-huma pi tuitary LH The LH preparation had a biological acti y of 14 000 IU (2nd IRP HMG) per mg. The results e e e pressed in ng per ml. One ng was equivalent to 83 ng of LER 907 in the immunoassay

LH in urine was measured by using human chorionic gonadotrophin (HCG) labelled with ^{125}I and rabbit anti HCG antibodies. The HCG preparation (Leo AB, Helsingborg, Sweden) had a biological acti ity of 13 000 IU (2nd Int. Stand. for HCG) per mg. The results were expressed in IU of the 2nd IRP for HMG per 24 hours

RESULTS

Estrogens

The total estrogen excretion before and after the menarche is shown in Table I and Fig. 1 The average 24-hour excretion of the girls with a bone age of 8–9 years was 4 6 μg/24 h with advanc ing bone age the excretion increased the mean value for the 13-year-old girls exceeding 11 μg/ 24 h. In menstruating girls the average level re mained the same up to 2 years after the men arche following which a steeper increase began until the mean excretion of girls who had men struated for 3 years had reached a level of 17 μg/24 h corresponding to an adult follicular phase level (20) The tandard deviation of the estrogen values in premenarcheal girls was dis tinctly smaller than that of the menstruating girls.

The values for estradiol estrone and estriol are also presented in Table I and Fig. 1 A survey of the columns showing the mean excretion reveals that the course of the estriol increase is very similar to that of the total estrogen increase. both show a gentle premenarcheal increase and

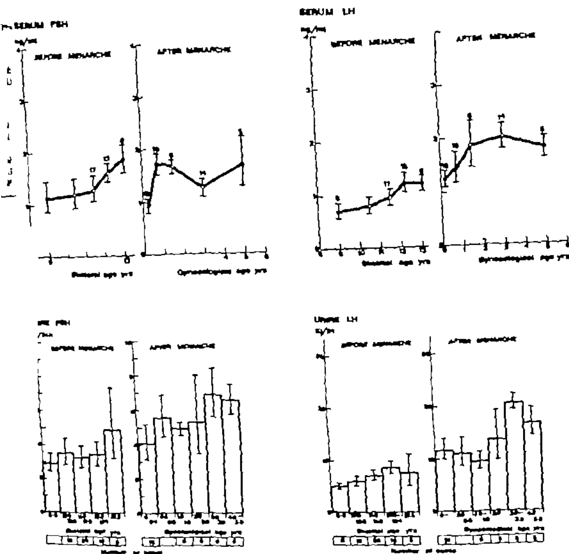


Fig. 5. Serum levels and urinary excretion of FSH and LH in pre- and postmenarcheal girls.

take place until immediately after the menarche, some 4 months later. The postmenstrual adult level, approx. 2 ng/ml, was reached after 24 months of menstruation. The difference between the means for premenarcheal (0.96 ng/ml) and postmenarcheal (1.62 ng/ml) groups was highly significant ($p < 0.001$).

The increase in urinary LH values (Fig. 5) before the menarche was considerably greater and no significant rise was associated with the menarche itself. A steeper rise took place some years after the menarche and the adult level was reached *after* 3 years of menstruation. The difference between the pre- and postmenarcheal values was highly significant ($p < 0.001$).

LH and FSH values vs. stage of puberty and skeletal age

Fig. 6 shows that the urinary LH values agreed well with the breast and pubic hair development ratings, as did serum LH values. The urinary FSH values followed to some extent the breast and pubic hair development ratings but not so closely as the urinary LH values. On the other

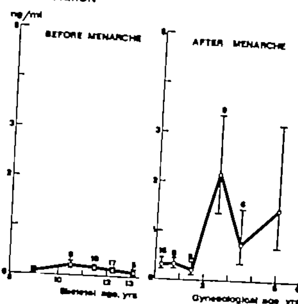


Fig 3 Serum levels of progesterone pre- and postmenarcheal girls.

archeal group, a large proportion of the readings approaches or equals zero. The mean value of these readings is 0.13 ng/ml. In the postmenarcheal group these same low levels were found even during the second year of menstruation. After the second postmenarcheal year the mean values increased suddenly A wide range in progesterone values, not seen in the premenarcheal group, was characteristic of the older girls. In the group of girls menstruating for ⩾2 years there were 7 values ⩾2 ng/ml.

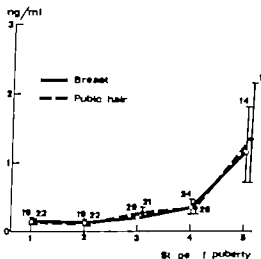


Fig 4 Serum levels of progesterone according to stages of puberty

Table IV *Serum levels of progesterone according to skeletal age ng/ml*

Skeletal age, y	*N*	Mean	Mean S.E.M.	*M*+ S.E.M
8–9.9	8	0.07	0.04	0.11
10–10.9	9	0.20	0.13	0.27
11–11.9	22	0.18	0.14	0.24
12–12.9	32	0.20	0.16	0.26
13–13.9	10	0.49	0.29	0.81
14–14.9	15	0.49	0.30	0.76
15–adult	8	1.27	0.93	1.77

Fig. 4 shows the distribution of the progesterone values according to the pubic hair and breast development ratings. It is evident that the mean serum progesterone levels increase steeply after rating 4 is reached. Table IV shows the progesterone levels according to the skeletal age.

FSH

In the youngest group (bone age 8–9 years) the mean serum value was 1.12 ng/ml (Fig 5). From a level of 1.26 ng/ml in the 12th year of bone age the mean value increases to 1.84 ng/ml by the menarche (13th year of bone age). A lower level of 0.92 ng/ml was recorded for the first 6 postmenarcheal months, and the adult level was again reached in one year (1.71 ng/ml).

The urinary excretion of FSH equalled the serum level (Fig. 5) An increase was noted about 3–4 years before the menarche, and a reduction during the first 6 postmenarcheal months. In the 1st to 3rd gynecological years the FSH level remained approximately constant at about 5.2 IU/24 h. In the 4th gynecological year the FSH values rose to 6.9 IU/24 h. The difference in serum FSH values between the pre- and postmenarcheal groups were not significant, whereas the difference in the urinary FSH values was significant ($p < 0.01$).

LH

The LH determinations were made on serum and 24-hour urine specimens taken during the same day as FSH. The serum values (Fig 5) showed that premenarcheally the LH rise was not particularly pronounced however from the 10th to 12th year of bone age the LH values rose from 0.79 to 1.21 ng/ml. The next rise after this does not

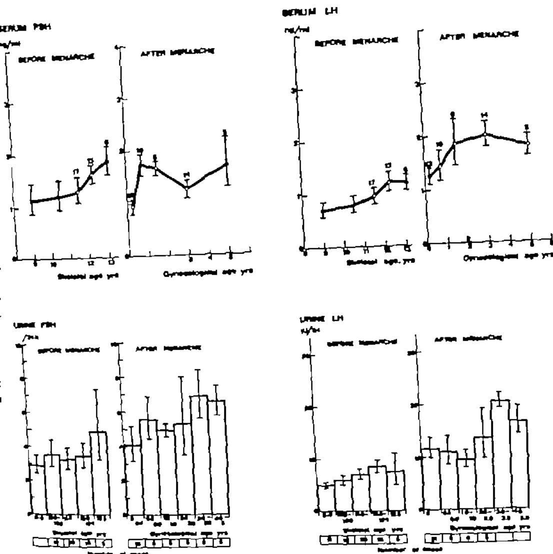


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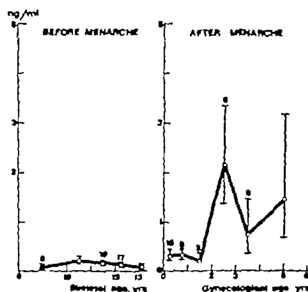


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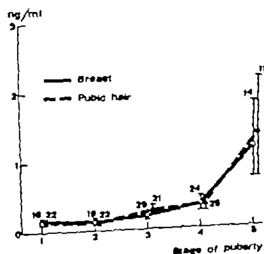


Fig 4 Serum levels of progesterone according to stages of puberty

Table IV *Serum levels of progesterone according to skeletal age ng/ml*

Skeletal age, y	*N*	Mean	Mean S.E.M	M+ S.E.M.
8–9 9	8	0.07	0 04	0.11
10–10.9	9	0.20	0.13	0.27
11 11 9	22	0.18	0.14	0.24
12 12.9	32	0.20	0.16	0.26
13–13 9	10	0.49	0.29	0.81
14–14 9	15	0.49	0.30	0.76
15–adult	8	1.27	0.93	1 77

Fig. 4 shows the distribution of the progesterone values according to the pubic hair and breast development ratings. It is evident that the mean serum progesterone levels increase steeply after rating 4 is reached. Table IV shows the progesterone levels according to the skeletal age.

FSH

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LH

The LH determinations were made on serum and 24 hour urine specimens taken during the same day as FSH. The serum values (Fig. 5) showed that premenarcheally the LH rise was not particularly pronounced however from the 10th to 12th year of bone age the LH values rose from 0.79 to 1 71 ng/ml. The next rise after this does not

Table V. *Serum levels of FSH according to skeletal age, ng/ml*

Skeletal age, y	N	Mean	M− S.E.M.	M+ S.E.M.
8–9.9	8	1.12	0.84	1.45
10–10.9	9	1.18	0.92	1.49
11–11.9	20	1.24	1.05	1.46
12–12.9	30	1.52	1.39	1.66
13–13.9	11	1.42	1.17	1.71
14–14.9	14	1.53	1.37	1.70
15–adult	8	0.98	0.87	1.11

Table VII. *Serum levels of LH according to skeletal age ng/ml*

Skeletal age, y	N	Mean	M− S.E.M.	M+ S.E.M.
8–9.9	8	0.70	0.56	0.85
10–10.9	9	0.79	0.65	0.96
11–11.9	20	0.94	0.80	1.08
12–12.9	30	1.18	1.09	1.28
13–13.9	11	1.66	1.36	2.01
14–14.9	14	2.05	1.80	2.33
15–adult	8	1.80	1.68	1.93

largely unknown. The beginning of the menstrual periods is always a definite and often drastic event in a girl's life. The correlation of age of the menarche to fertility and the age of menopause are approximate but important criteria in gynecological work.

During sexual maturation, estrogens have been considered the most important steroids affecting the gonadostat in women (17).

With the Adlercreutz et al. (1, 2) method used all the girls in the series had a detectable urinary estrogen excretion, i.e. girls with bone age of 8 years already had mean total estrogen excretion of nearly 5 μg/24 h. Since the purpose of the present study was primarily to trace the changes taking place around the time of the menarche, no younger girls were included in the series. Dewhurst (10), and Pennington & Dewhurst (31) showed, with an analogous method, that estrogen excretion could be demonstrated in girls as young as 3 years. The present results are in agreement with the findings of these studies and the study by Jenner et al. (17).

The classification we used, trying to combine individuals of the same biological maturity produced results divergent from earlier concepts: a slow increase rather than a rapid rise in the excretion of estrogen was demonstrable in our series. A particular finding is that in this study no premenarcheal multiplication of estrogen took place, contrary to what was reported e.g. by Nathanson et al. (28). A detailed analysis of the estrogen fractions in our study revealed that both estradiol and estrone increased significantly just before the menarche while estriol remained almost unchanged. In so far as the estradiol excretion reflects the estrogen of ovarian origin and estrone primarily that of adrenal origin, it is possible that the menarche is preceded by an increased activity in both ovaries and adrenals.

The total estrogen excretion was significantly correlated with the bone age and sexual development. According to Jenner et al. (17), the plasma estradiol values correlate well with bone age, chronological age and especially with the clinical evaluation of pubertal development. The present results are in accordance with those obtained by them.

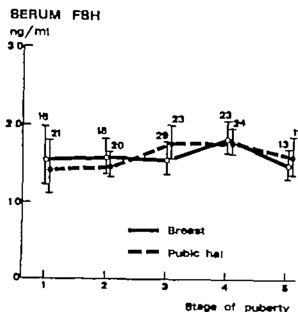
It seems as if the hormonal changes around the menarche were very small, smaller than has been assumed previously. After a steady level for about 18 months, counted from the men-

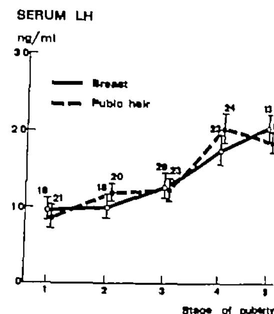
Table VI. *Urinary excretion of FSH according to skeletal age IU/24 h*

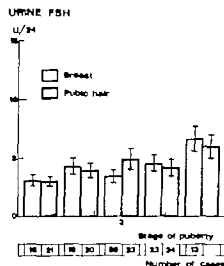
Skeletal age, y	N	Mean	M− S.E.M.	M+ S.E.M.
8–9.9	11	2.91	2.41	3.52
10–10.9	12	3.33	2.47	4.35
11–11.9	24	3.05	2.58	3.61
12–12.9	3	3.95	3.37	4.64
13–13.9	11	6.55	5.29	7.57
14–14	14	4.79	3.98	5.76
15–adult	8	6.58	5.77	7.50

Table VIII. *Urinary excretion of LH according to skeletal age IU/24 h*

Skeletal age, y	N	Mean	M− S.E.M.	M+ S.E.M.
8–9.9	11	4.94	4.39	5.55
10–10.9	12	5.87	5.13	6.72
11–11.9	24	6.70	5.94	7.56
12–12.9	32	9.84	8.74	11.08
13–13.9	14	13.34	10.70	16.64
14–14.9	15	11.78	10.25	1 .54
15–adult	6	15.41	12.53	18.95







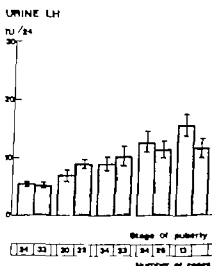


Fig 6 Serum levels and urinary excretion of FSH and LH according to stages of puberty

hand, serum FSH values showed no relationship with the degree of sexual maturity

Tables V–VIII show the urinary excretion and serum levels of the FSH and LH according to the skeletal age.

DISCUSSION

The menarche is more closely related to skeletal than to chronological age (25). In a cross-sectional study it is impossible to know how far a young girl is from the menarche that is to say the negative gynecological age" cannot be used, but the girls can better be grouped according to their stage of maturity on the basis of their skeletal rather than their chronological age, or by using the pubertal development scale according to Tanner (40). For this reason we decided to record the premenarcheal hormone excretion by groups based on the bone age The results show that this method produced reasonably small fluctuations in the values. In clinical work such normal values will probably prove useful.

The endocrine changes which led to sexual maturity and cyclic interaction between the central nervous system and the ovaries are still

the adult level until the age of about 18 years, that is, the 4th to 5th year of menstruation. Summarizing, it can be said that the postmenarcheal endocrine development continued at least to the age of 18. The premenarcheal development is probably also linear but an acceleration takes place at 8 years of age. From the results of present study it was not possible to attribute the onset of menarche to stimulation by any individual hormone.

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REFERENCES

1. Adlercreutz, H. & Luukkainen, T. Biochemical and clinical aspects of the enterohepatic circulation of oestrogens. Acta Endocrinol (Kbh.) Suppl. *1* 4 101, 1967.
2. Adlercreutz, H., Salokangas, A. & Luukkainen, T. Measurements of oestrogens in biological material. In The Gas Liquid Chromatography of Steroids. Proceedings of a symposium held at the University of Glasgow on 4 to 6 April 1966 (ed. J. K. Grant). University Press, Cambridge 1967 (p) (Memoirs of the Society for Endocrinology No. 16.)
3. Beling, C. G. Gel filtration of conjugated urinary oestrogens and its application in clinical assays. Acta Endocrinol (Kbh.) Suppl. 79, 1963.
4. Bergstrand, C. G. & Gemzell, C. A. Pregnanediol excretion in normal children and in children with various endocrine disorders, including congenital adrenal hyperplasia. J Clin Endocrinol *17* 870, 1957.
5. Blizzard, R. M., Johanson, A., Guyda, H., Baghdassarian, A., Raiti, S. & Migeon, C. J. Recent developments in the study of gonadotropin secretion in adolescence. *In* Adolescent Endocrinology (*ed.* F. P. Heald & W. Hung), p. 1. Butterworths, London, Appleton-Century-Crofts, New York, 1970.
6. Boyar, R., Finkelstein, J., Roffwarg, H., Kapen, S., Weitzman, E. & Hellman, L. Synchronization of augmented luteinizing hormone secretion with sleep during puberty. New Engl J Med 287 582, 1972.
7. Brown, J. B. A chemical method for determination of oestriol, oestrone and oestradiol in human urine. Biochem J 60 185, 1955.
8. Brown, P. S. Human urinary gonadotrophins. I. In relation to puberty. J Endocrinol *17* 329, 1958.
9. Bäckström, L. & Kantero, R.-L. Cross-sectional studies of height and weight in Finnish children aged from birth to 20 years. Acta Paediat Scand 60 Suppl. 220, 9, 1971.
10. Dewhurst, C. J. Gynaecological disorders of infants and children. F. A. Davis Co., Philadelphia, 1963.
11. Diczfalusy, E. & Lauritzen, Ch. Oestrogene beim Menschen. Springer-Verlag, Berlin, Gottingen and Heidelberg, 1961.
12. Fitschen, W. & Clayton, B. E. Urinary excretion of gonadotrophins with particular reference to children. Arch Dis Childh 40 16, 1965.
13. Frandsen, M. On the excretion of oestrogenic and androgenic substances in the urine of women. Acta Obstet Gynecol Scand 20 Suppl. 1, 1, 1940.
14. *Hayes, A. & Johanson, A. Excretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in urine by pubertal girls.* Pediat Res 6 18, 1972.
15. Huffman, J. W. The Gynecology of Childhood and Adolescence. W. B. Saunders Co., Philadelphia, London and Toronto, 1968.
16. Jayle, M. F. & Crépy, O. The excretion and measurement of urinary phenolsteroids. Ciba Found Coll Endocrinol 2 84, 1952.
17. Jenner, M. R., Kelch, R. P., Kaplan, S. L. & Grumbach, M. M. Hormonal changes in puberty. IV. Plasma estradiol, LH, and FSH in prepubertal children, pubertal females, and in precocious puberty, premature thelarche, hypogonadism, and in a child with a feminizing ovarian tumor. J Clin Endocrinol 34 521, 1972.
18. Johanson, A., Guyda, H., Light, C., Migeon, C. & Blizzard, R. Serum luteinizing hormone by radioimmunoassay in normal children. J Pediat 74 416, 1969.
19. Johansson, E. D. B. A simplified procedure for the assay of progesterone. Karolinska Symposia on Research Methods in Reproductive Endocrinology. 2nd Symposium. Acta Endocrinol (Kbh.), Suppl. 147 188, 1970.
20. Johansson, E. D. B., Wide, L. & Gemzell, C. Luteinizing hormone (LH) and progesterone in plasma and LH and oestrogens in urine during 42 normal menstrual cycles. Acta Endocrinol (Kbh.) 68 502, 1971.
21. Klopper, A., Michie, E. A. & Brown, J. A. A method for determination of urinary pregnanediol. J Endocrinol *12* 209, 1955.
22. Kulin, H. E., Rifkind, A. B., Ross, G. T. & Odell, W. D. Total gonadotropin activity in the urine of prepubertal children. J Clin Endocrinol 27 1123, 1967.
23. Lamberg, B. A., Kantero, R.-L., Saarinen, P. & Widholm, O. Thyroid hormones and thyrotrophin in the blood in female adolescents. Endocrine changes before and after menarche. III. Acta Endocrinol Scand (in press).
24. Lee, P. A., Midgley, A. R. & Jaffe, R. B. Regulation of human gonadotropins. VI. Serum follicle stimulating and luteinizing hormone determinations in children. Organon 31 243, 1970.

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arche all estrogen fractions rose smoothly until the 3rd gynecological year i.e. up to the age of about 16 years. The significance of the increase in the total estrogen values after the 3rd year of menstruation will be discussed below and compared with the serum progesterone levels.

The premenarcheal serum progesterone levels, from the 8th year of bone age onwards, are naturally very low and are often undetectable, being below the lower limit of the method used. There were great deviations in the serum progesterone values in the group of girls who had menstruated for over 2 years. Although all the specimens had been taken on the 7th to 10th day of the cycle, it is evident that the high values reflect either peri- or postovulatory levels. By 2 years after the menarche in only one girl did the serum progesterone reach at the adult peri- or postovulatory levels (≥2 ng/ml). The corresponding estrogen and gonadotrophin levels during this stage of development are below the corresponding adult level in the follicular phase, according to the results obtained in the same laboratory by Johansson et al (20). In the next age group of girls, menstruating for more than 2 years, 7 serum values were high (≥2 ng/ml). The parallel mean total estrogen level in this group was increased even more. The increased serum progesterone values can be explained in several ways. One definite possibility is that the girls mistook a small ovulation bleeding for menstruation. This explanation would fit the finding of high progesterone levels together with a high excretion of estrogens and a low serum FSH level.

The question as to whether the ovarian or the hypothalamo-hypophyseal hormone stimulation triggers off the menarche is still unclear. Reports in the literature on the LH/FSH ratio during puberty in girls vary a great deal and are difficult to compare because the standards used have been different. A certain estrogen stimulation is naturally necessary for a feedback system to be established. The extent to which this estrogen excretion is primarily ovarian and that to which it depends on adrenals, is not known. According to e.g. Penny et al. (32), and Rifkind et al (34) the FSH excretion in girls increases before that of LH. In the present series FSH at the age of 8 years was already at a low adult level, and the increase up to the menarche was very moderate. It seems as if a relatively major increase had taken place before the age of 8 years, which unfortunately was the youngest age group in the present series. According to Westphal & Wide (44), an adult level of FSH is reached by boys considerably later than by girls, and probably does not occur until spermatogenesis begins. Penny et al. (32) found a significant increase in serum FSH in 5–8-year-old girls, while the LH rise was not significant until 8–10 years of age. Yen et al. (51) found that LH was detectable in both girls and boys at the age of 8 years. By the age of 14 the LH rise in girls was reaching the adult level and, in their study was 6-fold up to sexual maturity. The corresponding increase in our series was 2 to 3-fold, which is in good accord with the results of Johanson et al. (18). A significant increase in LH excretion in our study was demonstrable 2 years before the menarche. Before the menarche the rise in 4 hour urinary LH excretion was more pronounced than the rise in the serum LH level. Peaks of LH in urine at the time of the menarche have been reported by Hayes & Johanson (14) and such irregular peaks of LH in urine were also found by Widholm & Kantero (49), using a daily Luteonosticon® test (Organon) on samples collected immediately after the menarche. These changes in the urinary excretion of LH might be explained by the nocturnal increases in secretion of LH at puberty reported by Boyar et al. (6). Jenner et al. (17) are of the opinion that the initial hormone event in female puberty is an increased excretion of gonadotrophins, especially FSH.

Some reports have mentioned a "battleship puberty" but from the endocrinological point of view sexual maturity seems to take place by moderate, almost linear increases of both gonadotrophins and steroids, starting before the age of 8 years. The values for the corresponding 17-KS and 17-OH-CS studies follow the premenarcheal estrogen curve (p. 201).

The results obtained indicate that the menarche is followed by steady hormone levels, both gonadotrophins and steroids remaining constant for 1– years. However the development of regular cyclical ovulations is probably a slow process. Widholm & Kantero (47) found irregular cycles in about 20% of girls with a 5-year history of menstruation. It is also seen from a study on thyroid function during puberty (23) from this series that the TSH excretion does not fall to

e adult level until the age of about 18 years, that is, the 4th to 5th year of menstruation. Summarizing, it can be said that the postmenarcheal endocrine development continued at least to the age of 18. The premenarcheal development is probably also linear but an acceleration takes place at 8 years of age. From the results of present study it was not possible to attribute the onset of menarche to stimulation by any individual hormone.

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REFERENCES

1 Adlercreutz, H. & Luukkainen, T Biochemical and clinical aspects of the enterohepatic circulation of oestrogens. Acta Endocrinol (Kbh Suppl 124 101, 1967

2 Adlercreutz, H Salokangas, A & Luukkainen, T Measurements of oestrogens in biological material In The Gas Liquid Chromatography of Steroids Proceedings of symposium held at the University of Glasgow on 4 to 6 April 1966 (ed. J K. Grant). University Press, Cambridge, 1967 (b). (Memoirs of the Society for Endocrinology No. 16)

3 Beling, C. G Gel filtration of conjugated urinary oestrogens and its application in clinical assays Acta Endocrinol (Kbh.), Suppl. 79 1963

4 Bergstrand, C G & Gemzell, C. A Pregnanediol excretion in normal children and in children with various endocrine disorders, including congenital adrenal hyperplasia. J Clin Endocrinol 17 870, 1957

5 Boyar, R M Johanson, A Gaydа, H Baghdassarian, A Raiti, S & Migeon, C J Recent developments in the study of gonadotropin secretion in adolescence I Adolescent Endocrinology (ed. F P Heald & W Hung), p 1 Butterworths, London, Appleton Century-Crofts, New York, 1970

6 Boyar R., Finkelstein, J Roffwarg, H Kapen, S Weitzman, E & Hellman, L Synchronization of augmented luteinizing hormone secretion with sleep during puberty New Engl J Med 287 582, 1972

7 Brown, J B A chemical method for determination of oestriol, oestrone and oestradiol in human urine Biochem J 60 185 1955

8 Brown, P S Human urinary gonadotrophins. I. In relation to puberty J Endocrinol 17 329 1958.

9 Bäckström, L. & Kantero, R. L. Cross-sectional studies of height and weight in Finnish children aged from birth to 20 years. Acta Paediat Scand 60 Suppl. 220: 9 1971

10 Dewhurst, C. J Gynecological disorders of infants and children. F A. Davis Co Philadelphia, 1963

11 Diczfalusy E. & Lauritzen, Ch.: Oestrogene beim Menschen. Springer Verlag, Berlin, Gottingen and Heidelberg, 1961.

12 Fitschen, W & Clayton, B E.. Urinary excretion of gonadotrophins with particular reference to children Arch Dis Childh 40 16, 1965

13 Forsbäck, M On the excretion of oestrogenic and androgenic substances in the urine of women. Acta Obstet Gynecol Scand 20 Suppl. 1 1, 1940.

14 Hayes, A & Johanson, A.. Excretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in urine by pubertal girls. Pediat Res 6 18, 1972.

15 Huffman, J W The Gynecology of Childhood and Adolescence W B. Saunders Co., Philadelphia, London and Toronto, 1968.

16. Jayle, M. F & Crépy O The excretion and measurement of urinary phenolsteroids. Ciba Found Coll Endocrinol 2 84 1952.

17 Jenner M R Kelch, R P Kaplan, S L. & Grumbach, M. M Hormonal changes in puberty IV Plasma estradiol, LH, and FSH in prepubertal children, pubertal females, and in precocious puberty premature thelarche, hypogonadism, and in child with feminizing ovarian tumor. J Clin Endocrinol 34 521 1972

18. Johanson, A Gayda, H Light, C., Migeon, C. & Blizzard, R Serum luteinizing hormone by radioimmunoassay in normal children J Pediat 74 416, 1969

19 Johansson, E D B A simplified procedure for the assay of progesterone Karolinska Symposia on Research Methods in Reproductive Endocrinology 2nd Symposium, Acta Endocrinol (Kbh), Suppl. 147 188, 1970

20 Johansson, E D B., Wide, L & Gemzell, C Luteinizing hormone (LH) and progesterone in plasma and LH and oestrogens in urine during 42 normal menstrual cycles. Acta Endocrinol (Kbh.) 68 502, 1971

21 Klopper A Michie, E A & Brown, J A A method for determination of urinary pregnanediol. J Endocrinol 12 209 1955

22. Kulin, H E Rifkind, A B Ross, G. T & Odell, W D Total gonadotropin activity in the urine of prepubertal children. J Clin Endocrinol 27 1123, 1967

23 Lamberg, B A., Kantero, R.-L. Saarinen, P & Widholm, O Thyroid hormones and thyrotrophin in the blood in female adolescents Endocrine changes before and after menarche. III. Acta Endocrinol Scand (in press)

24 Lee, P A R Midgley A A Jaffe R B Regulation of human gonadotropins. VI Serum follicle stimulating and luteinizing hormone determination in children. Orpinen 31 ..40, 1970

14 74261

25 Marshall, W A & Tanner J M Variations in the pattern of pubertal changes in girls. Arch Dis Childh 44 291 1969
26 McArthur J W., Ingersoll F M & Worcester J Urinary excretion of interstitial-cell stimulating hormone by normal males and females of various ages. J Clin Endocrinol 18 460, 1958
27 Midgley A R Jr Radioimmunoassay: A method for human chorionic gonadotropin and human luteinizing hormone Endocrinology 79 10 1966.
8 Nathanson, I T Towne, L. E. & Aub, J C. Normal excretion of sex hormones in childhood. Endocrinology 28 851 1941
29 Neill, J D Johansson, E. D B Datta J K & Knobil, E.. Relationship between the plasma levels of luteinizing hormone and progesterone during the menstrual cycle. J Clin Endocrinol 27 1167 1967
30 Odell, W D Ross, G T & Rayford, P L. Radioimmunoassay for luteinizing hormone in human plasma or serum. Physiologic studies. J Clin Invest 46 248 1967
31 Pennington, G W & Dewhurst, C. J Hormone excretion in premenarcheal girls. Arch Dis Childh 44 629 1969
32. Penny R., Guyda H J Baghdassarian, A Johanson, A J & Blizzard, R M Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development J Clin Invest 49 1847 1970
33 Procopé, B J Studies on the urinary excretion biological effects and origin of oestrogens in post-menopausal women. Acta Endocrinol (Kbh.), Suppl. 135, 1968.
34 Rifkind, A B., Kulin, H. E. & Ross, G T Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the urine of prepubertal children. J Clin Invest 46 19_5 1967
35 Roos, P Follicle stimulating hormone Acta Endocrinol (Kbh.), Suppl. 1 131 1 1968.
36. Saxena B B Demura H Candy H M. & Peterson, R. E. Radioimmunoassay of human follicle stimulating hormones in plasma. J Clin Endocrinol 3 519 1968
37 Schalch, D S., Parlow A. F Boon, R. C & Reichlin, S.. Measurement of human luteinizing hormone / plasma by radioimmunoassay J Clin Invest 47 665 1968
38 Sizonenko, P C. Evolution des concentrations sanguines des hormones lutéinisa t (LH) t folliculo-stimulante (FSH) chez la fille de la naissance à la puberté. Extraits des XXVe Assises françaises de Gynécologie, 1971 pp 45
39 Sizonenko, P C., Burr I M Kaplan, S. L. & Grumbach M M Hormonal changes in puberty II Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty and bone age in normal girls. Pediat Res 4 36, 1970.
40. Tanner J M.. Growth at Adolescence Blackwell Scientific Publications, O ford 196..
41 Tanner J M., Whitehouse, R. H & Healy M. J. R A new system for estimating skeletal maturity from the hand and wrist, with standards derived from a study of 2600 healthy British children. Part II. The scoring system. International Children's Centre, Paris, 1962.
42 Tiisala, R., Kantero, R. L., Bäckström, L. & Hallman, N A mixed longitudinal study on skeletal maturation in healthy Finnish children aged 1 to 5 years. Hum Biol 41 560 1969
43 Tiisala, R Kantero, R. L. & Tamminen, T A mixed longitudinal study on skeletal maturation in healthy Finnish children aged 5 to 10 years. Hum Biol 43 224 1971
44 Westphal, O & Wide, L.. To be published
45 Wide L. & Porath, J Radioimmunoassay of proteins with the use of sephadex-coupled antibodies. Biochim Biophys Acta 130 _57 1966.
46. Wide L. Radioimmunoassays employing immunosorbents. Acta Endocrinol (Kbh.), Suppl. 142:207, 1969
47 Widholm O & Kantero, R.-L A statistical analysis of the menstrual patterns of 8000 Finnish girls and their mothers. Acta Obstet Gynecol Scand 49 Suppl. _, 1970.
48 Widholm O Kantero R.-L., Johansson, E. D. B. & Wide L. An endocrinological study of the puberty Acta Obstet Gynec Scand 50 76, 1971
49 Widholm, O & Kantero R L. Daily excretion of LH and FSH in urine immediately after menarche In manuscript, 1973
50. Vollman, R F Patterns of menstrual performance in adolescent girls Proc. 2nd Wld. Congr Fertil. Steril Naples, 1956
51 Yen, S. S C Vicic, W J & Kearchner D V Gonadotropin levels i puberty I Serum luteinizing hormone. J Clin Endocrinol 29 382, 1969

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STUDY OF GLUCOSE-6-PHOSPHATE AND ISOCITRATE DEHYDROGENASES DNA, RNA AND TOTAL NITROGEN IN THE RABBIT PLACENTA DURING ITS HYPERTROPHIC RESPONSE TO OVARIECTOMY

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Abstract Glucose-6-phosphate dehydrogenase isocitrate dehydrogenase, DNA, RNA and total nitrogen were analyzed in the rabbit placenta during its hypertrophic response to ovariectomy. Isocitrate dehydrogenase activity was increased in the experimental placentae. Glucose-6-phosphate dehydrogenase, DNA, RNA and total nitrogen were unchanged.

The "luteo-placental shift" (LPS) in progesterone genesis occurs at different times in gestation and achieves different degrees of completion in laboratory mammals and the human (2). The human and the rabbit represent extreme species, in that in the human the LPS is completed as early as 3–4 weeks after the missed menstrual period (3) while in the rabbit it does not reach a biologically significant degree even during third trimester pregnancy. The rat is intermediate between these two extremes (2).

However, if rabbits are ovariectomized at around the 18th day of gestation and their progesterone deficiency is prevented by decremental progesterone (P) substitution therapy, the placentae undergo a compensatory hypertrophic response and sustain pregnancy after the cessation of P therapy (2).

The changes in dehydrogenase activities reflect metabolic requirements for NADPH in steroid synthesis. This type of biochemical change may occur in the hypertrophic placentae, in addition to weight increase (8). Whether or not this hypertrophic response is a compensatory effort in steroidogenesis can be examined by analyzing two dehydrogenases, glucose-6-phosphate dehydrogenase (G-6-PD E.C.1.1.1.49) and isocitrate dehydrogenase (ICD E.C.1.1.1.42), two major supernatant enzymes which produce NADPH for steroid synthesis. The analysis of DNA, RNA and total nitrogen can serve as a basis of examining this phenomenon.

MATERIAL AND METHODS

Fifteen rabbits (inbred in Orion, Mankkaa, Helsinki) were studied. The 8 Experimental animals were ovariectomized at day 18 of pregnancy under ether anesthesia and with strict aseptic precautions and given transient decremental progesterone treatment to sustain their pregnancy as described by Csapo (2). On the day of ovariectomy 4 mg progesterone, in oil, was given intramuscularly in two divided daily doses. Progesterone decreased by 1 mg every 2nd day and tapered off to the level of 0.5 mg/day on days 26–27 (2). The placentae were collected (by hysterectomy) at day 29, rapidly weighed and analyzed while still fresh. The seven Controls were intact pregnant rabbits, sacrificed at day 29.

G-6-PD and ICD

The placenta was homogenized in cold Krebs-Ringer solution and the homogenate ultracentrifuged 105 000 *g* for 30 min. The resulting high speed supernatant was used for assay. G-6-PD was measured at pH 7.5 and ICD at pH 9.0, at 37°C (5).

Total nitrogen, DNA, and RNA

The placenta was homogenized in cold 0.3 mol/1 KOH. After dilution, part of the homogenate was used for determining total nitrogen (6). The rest of the homogenate was hydrolyzed at 37°C for 20 hours, centrifuged and the supernatant neutralized in cold 0.5 N HCl, centrifuged, diluted, and its RNA content determined (7). The precipitate was dissolved in warm 0.5 N HCl and centrifuged. DNA was determined in the supernatant with diphenylamine-reaction (1). Calf thymus DNA (Calbiochem Co.) served as standard.

25 Marshall, W A & Tanner J M Variations in the pattern of pubertal changes in girls. Arch Dis Childh 44 291 1969

26 McArthur J W Ingersoll, F M. & Worcester J Urinary excretion of interstitial-cell stimulating hormone by normal males and females of various ages. J Clin Endocrinol 18 460, 1958.

27 Midgley A R Jr Radioimmunoassay: A method for human chorionic gonadotropin and human luteinizing hormone. Endocrinology 79 10, 1966.

8 Nathanson, I T Towne, L. E. & Aub J C., Normal excretion of sex hormones in childhood Endocrinology 28 851 1941

29 Neill, J D., Johansson E D B Datta J K & Knobil E. Relationship between the plasma levels of luteinizing hormone and progesterone during the menstrual cycle J Clin Endocrinol 27 1167 1967

30 Odell, W D Ross, G T & Rayford, P L. Radioimmunoassay for luteinizing hormone in human plasma or serum Physiologic studies. J Clin Invest 46 248 1967

31 Pennington, G W & Dewhurst C. J Hormone excretion in premenarcheal girls. Arch Dis Childh 44 629 1969

32 Penny R Guyda H J Baghdassarian, A Johanson, A J & Blizzard, R. M Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. J Clin Invest 49 1847 1970

33 Procopé B J Studies on the urinary excretion, biological effects and origin of oestrogens in post-menopausal women. Acta Endocrinol (Kbh.) Suppl. 135 1968

34 Rifkind, A B Kulin, H E. & Ross, G T Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the urine of prepubertal children. J Clin Invest 46 1925 1967

35 Roos, P.: Follicle stimulating hormone. Acta Endocrinol (Kbh.), Suppl. 1 131 1 1968

36. Saxena, B B Demura, H Candy H M & Peterson, R. E. Radioimmunoassay of human follicle stimulating hormones in plasma. J Clin Endocrinol 8 519 1968

37 Schalch, D S., Parlow A F., Boon, R C. & Reichlin, S Measurement of human luteinizing hormone in plasma by radioimmunoassay J Clin Invest 47 665 1968

38 Sizonenko, P C. Evolution des concentrations plasmatiques des hormones lutéinizante (LH) et folliculo-stimulante (FSH) chez la fille de la naissance à la puberté. Extraits des XXV Assises françaises de Gynécologie 1971 pp. 45

39 Sizonenko, P C., Burr I M., Kaplan, S. L. & Grumbach, M M Hormonal changes in puberty II. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty and bone age in normal girls. Pediat Res 4 36, 1970.

40. Tanner J M Growth at Adolescence Blackwell Scientific Publications, Oxford, 1962.

41 Tanner J M Whitehouse R. H & Healy M. J R. A new system for estimating skeletal maturity from the hand and wrist, with standards derived from a study of 2 600 healthy British children Part II The scoring system. International Children's Centre, Paris, 1962.

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43 Tiisala R. Kantero R L. & Tamminen T A mixed longitudinal study on skeletal maturation in healthy Finnish children aged 3 to 10 years. Hum Biol 43 224 1971

44 Westphal, O & Wide, L. To be published.

45 Wide L. & Porath, J Radioimmunoassay of proteins with the use of sephadex-coupled antibodies Biochim Biophys Acta 130 257 1966.

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47 Widholm O & Kantero, R.-L. A statistical analysis of the menstrual patterns of 8 000 Finnish girls and their mothers. Acta Obstet Gynecol Scand 49 Suppl. 4, 1970

48 Widholm, O Kantero R.-L. Johansson, E. D. B. & Wide L An endocrinological study of the puberty Acta Obstet Gynec Scand 50 76 1971

49 Widholm O & Kantero, R L. Daily excretion of LH and FSH in urine immediately after menarche. In manuscript, 1973

50 Vollman, R F Patterns of menstrual performance in adolescent girls. Proc 2nd Wld Congr Fertil Steril Naples, 1956

51 Yen S S. C Vicic, W J & Kearchner D V Gonadotropin levels in puberty I Serum luteinizing hormone. J Clin Endocrinol 29 382, 1969

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Table I *Placental weights, G-6-PD ICD DNA RNA and total N concentrations in rabbit placentae after ovariectomy and transient decremental progesterone treatment (mean ± S E.)*

In parentheses the number of placentae studied

	Control	Experimental
Placental weight, g	5.3 ± 0.03 (47)	6.2 ± 0.07 (37)
ICD/g, IU	3.5 ± 0.11 (12)	4 1 ± 0.17 (13)
ICD/DNA mg	1 4 ± 0.06 (11)	1.9 ± 0.13 (12)
G-6-PD/g, IU	670 ± 39 (11)	640 ± 84 (13)
G-6-PD/DNA mg	70 ± [illegible]2 (10)	[illegible]90 ± 46 (12)
DNA mg/g	2.5 ± 0 12 (25)	2.8 ± 0.15 (17)
RNA mg/g	1.[illegible] ± 0 05 ([illegible]8)	1.[illegible] ± 0.04 (25)
Total N mg/g	4.8 ± 0 10 (29)	4 9 ± 0.08 (25)

RESULTS

The experimental placentae were on average 17% larger than the controls ($P < 0.05$) ICD activity was increased in the experimental placentae ($P < 0.01$) The G-6-PD DNA RNA and total nitrogen were unchanged (Table I)

DISCUSSION

The maintenance of pregnancy in ovariectomized animals depends on the success of the compensatory changes in the placenta (4) Ovariectomy by inducing an increase in placental ICD activity provides the placenta with more NADPH for progesterone synthesis. Sustained progesterone synthesis permits the pregnancy to continue till term.

The increase in placental weight is apparently due to hypertrophy rather than to hyperplasia as indicated by unchanged DNA. These results explain some of the metabolic changes in the placenta during its hypertrophic response

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REFERENCES

1 Burton, K. Biochem J 6[illegible] 315 1956.
2. Csapo, A. I Postgrad Med J 45 57 1969
3 Csapo A. I., Ruttner B Sauvage J P Wiest, W G & Pulkkinen, M O Amer J Obstet Gynecol 11[illegible] 106[illegible], 1972.
4 Csapo, A. I. & Wiest, W G Endocrinology 85 735, 1969
5 King, J Practical Clinical Enzymology pp. 59 and 76 Van Nostrand, 1965.
6. Munari, O & Zilversmidt, D B. Anal Biochem 6, 370, 1963
7 Munro, H. N Hutchison, T R., Ramaiah, T R & Neilson, F J Br J Nutr 16, 387 196[illegible]
8. Pulkkinen, M. O & Csapo, E. J Reprod Fert 18 1[illegible]5 1969

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OVARIAN MORPHOLOGY IN EARLY AND LATE HUMAN PREGNANCY

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Abstract Representative ovarian biopsies ere obtained from 30 pregnant women aged 15 to 40 Group I consisted of 6 omen ith pregnancies of gestational age of 9 to 16 weeks, and group II consisted of 24 women ith pregnancies of gestational age of 36 to 42 weeks

The number of primordial follicles varied considerably in both groups, but seemed to be mainly dependent on the age of the individual oman W did not examine the follicular development before the 9th eek of pregnancy but at least from that time normal development of follicles up to the stage of Graafian follicles was observed in the great majority of omen.

A typical decidual reaction in the ovarian cortex as found in all women in group II, but this reaction may also occasionally occur ery early in pregnancy For instance, we observed marked decidual reaction in one woman in the 9th eek of pregnancy The decidual reaction was always located beneath or adjacent to the surface epithelium, and mostly in as of the nodular form, but it might also occur as single cells or as more diffuse and confluent formations of decidual cells In all cases of typical decidual reaction, the macroscopic appearance of the ovary as characteristic, the ovarian surface presented tiriform appearance due to network of thin, pale red ridges and small nodules An interesting observation in group I was that the decidual reaction in all cases seemed to be preceded by the existence of nodules or more confluent areas characterized by loose, proliferative connecti tissue containing many fibroblasts and capillaries

The most constant finding in both groups as the proliferation and luteinization of the theca cells surrounding both the growing and atretic follicles. However it should be emphasized that these luteinized theca cells ere only found around antral and Graafian follicles and atretic follicles of similar size but never around even the largest primary follicles

W are not convinced of the significance of the previously described so-called surface cell proliferation, even if similar changes ere observed in 57% of the 30 omen investigated These changes ere mainly observed in areas here the epithelium covered depressed or retracted parts of the ovarian stroma On the other hand, the prominent decidual nodules were covered by thin layer of flattened epithelial cells W therefore believe that the changes in the surface epithelium is merely an unspecific phenomenon.

The histology and function of the human ovary especially in the fertile period, have been extensively studied for many years. Recent advances in cytochemistry and an increasing knowledge of special staining techniques have revived interest in ovarian histology Many problems have been solved, but there remain several unanswered questions regarding the effect of pregnancy on ovarian morphology and function.

In pregnancy there is long-term exposure of the ovary to an unusual steroidal and gonadotropic environment. The morphologic responses to this stimulation are of considerable interest. So far most attention has been paid to the structure of the corpus luteum at different stages of pregnancy (1 3 6 7 8 13 16, 19) but information about the remaining ovarian structures is surprisingly sparse and reveals several discrepancies. The disparate observations may at least partly be explained by the small number of ovaries studied in the majority of investigations. However certain changes in ovarian morphology seem to be characteristic of pregnancy

The ovary at term shows some distinctive macroscopic and microscopic changes. The gross alteration consists of a network of pale reddish ridges over the surface of the ovary which microscopically is shown to be a decidual reaction located just beneath the surface epithelium (5 9 11 12, 13 14 16, 17). Another characteristic feature of the ovary during pregnancy is the more or less marked proliferation and luteinization of the theca cells surrounding both developing and atretic follicles (4 11 13 16) In addition, Israel

Table I *Data and ovarian morphology in group I*

Patient no	Age	Gestational age (weeks)	Primordial foll.	Primary foll	Antral foll.	Graafian foll	Corpus luteum fresh	Corpus luteum old	Atretic foll.	Follicular cysts	Stroma reaction	Proliferation of surface epithelium	Luteinization of theca cells around normal foll	Luteinization of theca cells around atretic foll.
1	36	9	+ +	+	+	+	−	+	+	+	Slight	+	+	+
2	32	9	+ +	+	−	+	−	+	+	+	Slight	−	+	+
3	15	18	+ + +	+	+	−	+	−	+	−	Decidua	+	+	
4	37	11	+	−	−	+	−	−	+	+	Slight	+	+	+
5	40	9	+	+	+	−	−	+	+	+	Decidua	−	+	+
6	37	14	+ +	+	+	+	+	+	+	+	Slight	+	+	+

et al. (9) and Maqueo & Goldzieher (11) have described a proliferation of the surface epithelial cells, but this finding has not been confirmed by other investigators. The very interesting problem of follicular development during pregnancy has only been sufficiently investigated by Govan (4) Finally Maqueo & Goldzieher (11) have described fibrosis of the cortical stroma in 22 % of their cases, but no other investigators have observed similar changes.

The purposes of the present study were 1) to investigate the development of ovarian follicles during pregnancy 2) to examine when the decidual reaction in the ovarian cortex starts, 3) to investigate when the proliferation and luteinization of the theca cells start, and whether these changes are found around follicles of all stages, and finally 4) to examine whether a proliferation of the surface epithelial cells is a common and specific finding during pregnancy

Table II *Data and ovarian morphology in group II*

Patient no.	Age	Gestational age (weeks)	Primordial foll.	Primary foll.	Antral foll.	Graafian foll.	Corpus luteum fresh	Corpus luteum old	Atretic foll	Follicular cysts	Stroma reaction	Proliferation of surface epithelium	Luteinization of theca cells around normal foll.	Luteinization of theca cells around atretic foll
7	30	40	+ +	+	+	+	+	+	+		Decidua	−	+	+
8	24	42	+ + +	+		+	−	−	+	−	Decidua	−	+	+
9	23	36	+ + +			+	+	−	+		Decidua	+	+	+
10	28	38	+ + +	+	+	+	−	−	+	+	Decidua		+	+
11	24	42	+ + +	+	+	+	+	+	+	+	Decidua		+	+
12	29	41	+ +	+	+	+	+	+	+		Decidua		+	+
13	30	39	+ +	+		+		+			Decidua		+	+
14	27	41	+ +	+	+		−				Decidua		+	+
15	29	39	+ +	+	−	+				+	Decidua	+	+	+
16	28	40	+ +	+	−	+				+	Decidua		+	+
17	29	41	+ +	+	+	+					Decidua		+	+
18	22	37	+ + +		−	+	−		+	+	Decidua		+	+
19	31	40	+ +	+	+		+		+	+	Decidua		+	+
20	26	41	+ +	+	−	+					Decidua		+	+
21	24	42	+ + +	+	+	+					Decidua	−	+	+
22	32	37	+ + +	+	−	−		+	+	+	Decidua	−		+
23	28	41	+ +	+	−	+				+	Decidua			+
24	25	41	+ +	+	−	+	+	−	+	+	Decidua		+	+
25	32	38	+ +	+	+	−			+		Decidua	+		+
26	24	41	+ + +	+	−	+	−		+		Decidua		+	+
27	38	41	+	+	−	+	+	+	+	+	Decidua			+
28	30	40	+	+	−	+	−	+			Decidua	+	+	+
29	34	40	+ + +	+	+	+	+	+	+		Decidua	+		+
30	20	40	+ + +	+	+	+	−	−	+	+	Decidua	−		+



Fig. 1. Ovarian biopsy from a 36-year-old woman (no. 1) in the 9th week of pregnancy showing an antral follicle surrounded by luteinized theca cells (HE, ×63).

MATERIAL AND METHODS

Representative ovarian biopsies were obtained by laparotomy from 30 women with intra-uterine pregnancies, who had previously had normal menstruation. It should be noted that the intention was to take the biopsy remote from the corpus luteum of pregnancy as we are especially interested in follicular development. In several cases, however, the biopsy included a smaller part of the corpus luteum too. The material was divided in two groups. Group I consisted of 6 women aged 15 to 40 (average 33), who had obtained permission for legal abortion and in 5 cases also for legal sterilization. The gestational age in group I varied from 9 to 18 weeks (average 12 weeks). Group II consisted of 4 women aged 20 to 38 (average 28), who had caesarean section performed for various reasons. The gestational age in group II ranged from 36 to 42 weeks (average 40 weeks). The specimens were fixed in formalin, blocked, sectioned and stained with hematoxylin-eosin and the van Gieson method.

The follicles were classified as primordial follicles, primary follicles, antral follicles, Graafian follicles and atretic follicles according to the following nomenclature. A primordial follicle consists of an oocyte surrounded by a single layer of flattened spindle-shaped cells. The term primary follicle applies to all follicles from the moment when the flat spindle-shaped cells around the oocyte become cuboidal and until the moment when the first small fluid-filled cleft-like spaces appear among the multilayered granulosa cells. When these spaces become confluent, the follicle has reached the stage of an antral follicle, and finally the follicle enlarges and matures to a Graafian

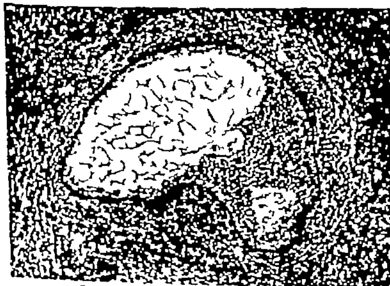


Fig. 2. Ovarian biopsy from a 30-year-old woman (no. 7) in the 40th week of pregnancy showing an antral follicle surrounded by luteinized theca cells (HE, ×63).

Table I *Data and ovarian morphology in group I*

Patient no	Age	Gestational age (weeks)	Primordial foll	Primary foll.	Antral foll	Graafian foll.	Corpus luteum fresh	Corpus luteum old	Atretic foll.	Follicular cysts	Stroma reaction	Proliferation of surface epithelium	Luteinization of theca cells around normal foll.	Luteinization of theca cells around atretic foll
1	36	9	++	+	+	+	−	+	+	+	Slight	+	+	+
2	32	9	++	+	−	+	−	+	+	+	Slight	−	+	+
3	15	18	+++	+	+	−	+	−	+	−	Decidua	+	+	+
4	37	11	+	−	−	+	−	−	+	+	Slight	+	+	+
5	40	9	+	+	+	−	−	+	+	+	Decidua	−	+	+
6	37	14	++	+	+	+	+	+	+	+	Slight	+	+	+

et al. (9) and Maqueo & Goldzieher (11) have described a proliferation of the surface epithelial cells, but this finding has not been confirmed by other investigators. The very interesting problem of follicular development during pregnancy has only been sufficiently investigated by Govan (4). Finally Maqueo & Goldzieher (11) have described fibrosis of the cortical stroma in 22% of their cases, but no other investigators have observed similar changes.

The purposes of the present study were 1) to investigate the development of ovarian follicles during pregnancy, 2) to examine when the decidual reaction in the ovarian cortex starts, 3) to investigate when the proliferation and luteinization of the theca cells start, and whether these changes are found around follicles of all stages, and finally 4) to examine whether a proliferation of the surface epithelial cells is a common and specific finding during pregnancy.

Table II *Data and ovarian morphology in group II*

Patient no.	Age	Gestational age (weeks)	Primordial foll.	Primary foll.	Antral foll.	Graafian foll.	Corpus luteum fresh	Corpus luteum old	Atretic foll.	Follicular cysts	Stroma reaction	Proliferation of surface epithelium	Luteinization of theca cells around normal foll.	Luteinization of theca cells around atretic foll.
7	30	40	++	+	+	+	+	+	+	+	Decidua	−	+	+
8	24	42	+++	+	−	+	−	−		−	Decidua	−	+	+
9	23	36	+++			+	+	−	+		Decidua	+	+	+
10	28	38	+++	+	+	+		−	+	+	Decidua		+	+
11	24	42	++	+	+	+	+	+	+	+	Decidua		+	+
12	29	41	++	+	+	+	+	+			Decidua		+	+
13	30	39	++	+		+	−				Decidua	+	+	+
14	27	41	++	+	+	−	−	+			Decidua	+	+	+
15	29	39	++	+		+		+			Decidua	+	+	+
16	28	40	+	+	−	+				+	Decidua		+	+
17	29	41	+	+	+	+					Decidua		+	+
18	22	37	+++			+			+		Decidua	−	+	+
19	31	40	++	+	+	+	+	+	+		Decidua		+	+
20	26	41	++	+	−	+					Decidua		+	+
21	24	42	+++	+	+	+	+				Decidua		+	+
22	32	37	+++	+	−	−		+	+	+	Decidua			+
23	28	41	++	+	−	+			+	+	Decidua	+		+
24	25	41	++	+	−	+	+			+	Decidua	+		+
25	32	38	++	+	+	−	−				Decidua		+	+
26	24	41	+++	+	−	+	+		+	+	Decidua		+	+
27	38	41	+	+	−	+	−	+			Decidua	+	+	+
28	30	40	+	+	−	+	+	+	+		Decidua	+	+	+
29	34	40	+++	+	+	+	−	−	+		Decidua	−		+
30	20	40	+++	+	+	+								



Fig. 5. Ovarian biopsy from 32-year-old woman (no. 2) in the 9th week of pregnancy showing an area of loose proliferative connective tissue with many fibroblasts and capillaries but without any decidual reaction and covered by flattened epithelium (HE, 63).

the two groups revealed both corpora albicantia and small follicular cysts, and all of them showed atretic follicles of different size. We did not observe fibrosis of the cortical stroma in any case.

The macroscopic appearance of the ovary at term was very characteristic. In all women in group II the ovarian surface presented a gyriform appearance because of a network of thin, pale red ridges and small nodules. These structures bled easily even on gentle pressure. Similar macroscopic changes were not observed in group I except in one case (no. 5). These macroscopic features of the ovarian surface were shown to result from a specific decidual reaction, located beneath or adjacent to the surface epithelium. The decidual reaction occurred in different forms. The nodular form was the most common variety but the reaction might also occur as single cells or as more diffuse and confluent formations of decidual cells. All 24 women in group II revealed a decidual reaction, and Fig. 4 shows a typical example of the nodular type. The characteristic finding in group I was areas of loose proliferative connective tissue with many fibroblasts and capillaries but without any real decidual reaction and covered by a flattened surface epithelium (Fig. 5).



Fig. 6. Ovarian biopsy from 40-year-old woman (no. 5) in the 9th week of pregnancy showing typical decidual reaction just beneath the flattened epithelium (HE, 105).



Fig. 3. Ovarian biopsy from a year-old woman (no. 30) in the week of pregnancy showing the cumulus and oocyte of a Graafian follicle (HE, × 105).

follicle. Atresia may occur at any stage of follicular development, and all these degenerating follicles are called atretic follicles. The number of primordial follicles was estimated approximately as few (+), several (+ +) or many (+ + +), whereas the more developed follicles were only recorded as present or not present.

The ovarian biopsies wer also examined for fresh corpora lutea, corpora albicantia, follicular cysts and perifollicular fibrosis. In addition special attention was paid to the possible existence of decidual reaction in the cortex, proliferation and luteinization of the theca cells surrounding follicles in all stages of development and proliferation of the surface epithelial cells.

FINDINGS

Tables I and II show the histologic observations in groups I and II respectively. The number of primordial follicles varied considerably in groups, but seemed to be mainly dependent on age of the individual woman. All women e one (no. 4) showed primary follicles in diff stages of development. It appears from the 2 columns in Tables I and II that 15 of th women revealed antral follicles (Figs. 1 and while 25 of the 30 women showed typical Gr ian follicles (Fig. 3) and in addition, t seemed to be no significant difference in follic development in the two groups. The finding only 11 of the 30 women showed fresh corp lutea might seem surprising, but is fully explai by the fact that we intended as previously m tioned, to take the biopsy outside the corpus teum. As expected, the majority of women

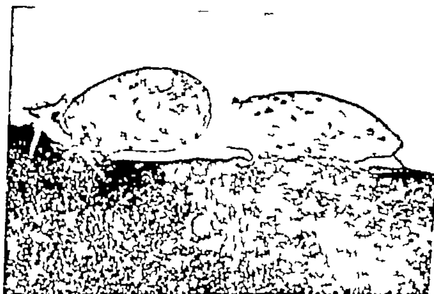


Fig. 4. Ovarian biopsy from -year-old woman (no. 1) in the 41st week of pregnancy showing prominent decidual nodules covered by flattened epithelium (HE, × 57).

and atretic follicles of similar size, but never around even the largest primary follicles. This also explains why patient no. 22, in contrast to all the other women, did not show any luteinization around the normal follicles.

DISCUSSION

Govan (4) has observed that until about the 10th week of pregnancy the cyclical development of follicles seemed to be suspended, and the ovary remained in the same structural state as it was around the time of ovulation. In spite of the increasing level of human chorionic gonadotropin (HCG) no new follicles appeared, and the mature follicles, which had not ovulated, persisted but showed gradual deterioration. After 10 weeks of gestation, however many new antral and Graaf ian follicles developed. In the present investigation we did not examine follicular development before the 9th week of pregnancy but at least from that time we found normal development of follicles up to the Graafian follicle stage.

Unfortunately very little is known about the plasma levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) during pregnancy Determinations of immunoreactive FSH in maternal serum during pregnancy have shown considerable variations. Consistently low values of FSH were observed by Jaffe et al. (10) and Parlow et al (15), whereas Faiman et al. (2) found the level of FSH in pregnancy similar to the highest level observed during the normal menstrual cycle Until lately it has not been possible to determine LH during pregnancy since no method has been available which could distinguish between LH and HCG Recently however Vaitukaitis et al. (18) have been able to produce specific antisera capable of making this distinction, and it should now be possible to determine the level of LH during pregnancy It will be most interesting to correlate the levels of FSH and LH to the follicular development at different stages of pregnancy

The decidual reaction in the ovarian cortex has been reported to be one of the most consistent changes during the last trimester of pregnancy (3 9 11 1 13 14 16, 17). This finding was confirmed by the present investigation, as we found decidual reaction in all 4 women in group II More interesting w the observation that the typical decidual reaction seems to be preceded by the existence of nodules or more confluent areas characterized by loose, proliferative connective tissue containing many fibroblasts and capillaries. These changes were found in all 6 women in group I, i.e. from the 9th week of gestation. It should be noticed that one of the women in group I showed a typical decidual reaction already at the 9th week of pregnancy but this finding was an exception. Little is known concerning the cause of the decidual reaction in the ovary during preg nancy but the reaction is probably due to a stimulation of the ovarian stroma either by HCG or by progesterone.

Another consistent finding in the ovary during pregnancy is the proliferation and luteinization of the theca cells surrounding both the growing and atretic follicles (4 11 13, 16). This very characteristic feature of the ovary was observed in all women investigated in the present study i.e. from the 9th week of gestation and throughout pregnancy It should be emphasized, however that these luteinized theca cells were only found around the antral and Graafian follicles and the atretic follicles of similar size, but never around the smaller growing follicles. It seems likely that this reaction of the theca cells is also caused by HCG stimulation, but the problem is still unsolved.

Finally we are not convinced of the significance of the surface cell proliferation previously described by Israel et al. (9) and Maqueo & Goldzieher (11). In 57% of the women investigated w observed similar changes of the surface epithelium locally In these areas the epithelial cells seemed to be columnar and crowded, which gav the surface fringy appearance It was remark able however that these changes were mainly observed in areas where the epithelium covered depressed or retracted parts of the ovarian stroma. On the other hand, the prominent decidual nodules were covered by very thin layer of flat tened epithelial cells. For this reason we believe that the so-called prolif ration of surface epithelial cells is merely a non-specific phenomenon.

REFERENCES

1. Brewer J. I. Studies of the human corpus luteum. Evidence for early onset of regression of the corpus luteum of menstruation. Amer J Obstet Gynecol 44 1048, 1942.



Fig. 7. Ovarian biopsy from a ... year-old woman (no. 26) in th... week of pregnancy showing "p... feration" of the surface epithe... cells (HE, × 105).

However it was interesting to observe an incipient decidual reaction in one woman (no. 3) in the 18th week of gestation, and even a typical decidual reaction in one woman (no. 5) as early as the 9th week of pregnancy (Fig. 6).

In the non pregnant woman of fertile age the surface epithelial cells form a single layer of cuboidal cells. We found the surface epithelium unchanged in 13 of the 30 pregnant women investigated, but in the remaining 17 women the epithelium showed certain changes. In some areas the epithelial cells seemed to be columnar and crowded which gave the surface a fringy appearance, but we did not observe a really m... layered epithelium in any case (Figs. 7 and 8).

The most consistent and characteristic fi... in the ovary throughout pregnancy seemed t... marked proliferation and luteinization of ... theca cells surrounding both the growing ... atretic follicles. This specific reaction must ... very early in pregnancy since it was obser... in all 6 women in group I, and generally it ... mained unchanged until term (Figs. 1 and ...). Furthermore we made the interesting observati... that this luteinization of the theca cells only o... curred around the antral and Graafian follicl...

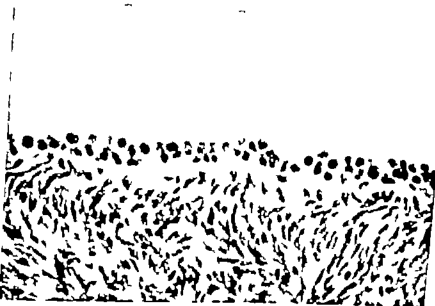


Fig. 8. Ovarian biopsy from ... 34-year-old woman (no. ?9) in the 40th week of pregnancy showing "proli-feration" of the surface epithelial cells (HE, × 105).

and atretic follicles of similar size, but never around even the largest primary follicles. This also explains why patient no. 22, in contrast to all the other women, did not show any luteinization around the normal follicles.

DISCUSSION

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Finally we are not convinced of the significance of the surface cell proliferation previously described by Israel et al. (9) and Maqueo & Goldzieher (11) In 57% of the women investigated we observed similar changes of the surface epithelium locally In these areas the epithelial cells seemed to be columnar and crowded, which gave the surface a fringy appearance. It was remarkable, however that these changes were mainly observed in areas where the epithelium covered depressed or retracted parts of the ovarian stroma. On the other hand, the prominent decidual nodules were covered by a very thin layer of flattened epithelial cells. For this reason we believe that the so-called proliferation of surface epithelial cells is merely a non-specific phenomenon

REFERENCES

1. Brewer J I Studies of the human corpus luteum. Evidence for early onset of regression of the corpus luteum of menstruation. Amer J Obstet Gynecol 44 1048, 1942

2. Faiman C., Ryan R. J Zwirek, S. J & Rubin, M E., Serum FSH and HCG during pregnancy J Clin Endocrinol 28 1323 1968.
3 Gillman, J & Stein, H B Human corpus luteum of pregnancy Surg Gynecol Obstet 72 129 1941
4 Govan, A D T., The human ovary in early pregnancy J Endocrinol 40 421 1968
5 Greene, R R & Nelson W W., Decidual reaction in the ovary Quart Bull NWest Univ Med Sch (Chicago) 26 197 1952.
6. Hertig, A T & Rock J Two human ova of pre villous stage having ovulation age of about 11 and 1 days respectively Contr Embryol 29 127 1941
7 — Two human ova of pre villous stage having developmental age of about 7 and 9 days respectively Contr Embryol 33 65 1945
8 — Two human ova of pre-villous stage having developmental age of about 8 and 9 days respectively Contr Embryol 33 169 1949
9 Israel, S L., Rubenstone, A & Meranze D The ovary at term. I. Decidualike reaction and surface cell proliferation. Obstet Gynecol 3 399 1954
10. Jaffe R. B Lee P A & Midgley A R., Jr Serum gonadotropins before at the inception of and following human pregnancy J Clin Endocrinol 29 1281 1969
11 Maqueo, M & Goldzieher J W Hormone induced alterations of ovarian morphology Fertil Steril 17 676, 1966.
12. Nelson, W W & Greene R R The human ovary in pregnancy Surg Gynecol Obstet 97 1 1953
13 — Some observations on the histology of the human ovary during pregnancy Amer J Obstet Gynecol 76 66, 1958
14 Novak, E. R & Woodruff, J D Gynecologic and Obstetric Pathology With Clinical and Endocrine Relations, 6th ed., p. 300 W B Saunders Company Philadelphia, 1967
15 Parlow A. F Daane, T A. & Dignam, W J On the concentration of radioimmunoassayable FSH circulating in blood throughout human pregnancy J Clin Endocrinol 31 213 1970.
16 Sauramo, H Histology and function of the ovary during the fertile period with special reference to the effect of pregnancy Acta Obstet Gynecol Scand 33 (Suppl. 2) 85 1954
17 Scott, R. B Endometriosis and pregnancy Amer J Obstet Gynecol 47 608, 1944
18. Vaitukaitis, J L., Braunstein, G D & Ross, G. T A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Amer J Obstet Gynecol 113 751 1972.
19 White, R F Hertig, A. T Rock, J & Adams, E Histological and histochemical observations on the corpus luteum of human pregnancy with special reference to corpora lutea associated with early normal and abnormal ova. Contr Embryol 34 55, 1951.

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PHOSPHOLIPIDS IN AMNIOTIC FLUID

I Relationship of Phospholipid Concentrations to Gestation Maternal Disease and Fetal Outcome

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Abstract Phospholipid concentrations were measured in 118 samples of amniotic fluid throughout the last trimester. In 112 of these, all six fractions were measured including total phospholipid content. The findings are correlated with gestational age, maternal disease and fetal outcome. Lecithin levels normally start to rise sharply from 34 weeks. Respiratory distress syndrome is shown to be associated with abnormally low concentrations of lecithin and lecithin sphingomyelin ratios below 2.0

The recognition of the high risk mothers whose prematurely born infants have a high incidence of respiratory distress syndrome (5 6) and the subsequent report of low lecithin levels in amniotic fluid of premature infants who develop respiratory distress syndrome following birth (7) has been followed by a number of studies of lecithin and sphingomyelin levels in amniotic fluid and their relationship to gestational age or infant disease (1 3 4 8 9 10). Although the concentrations of lecithin, the ratio of lecithin to sphingomyelin, the methods of extraction and the methods of lecithin determination were different in the different studies, the amniotic fluid of infants who develop RDS consistently showed low concentrations of lecithin and low lecithin/sphingomyelin ratios.

The present study was undertaken to determine the relationship of six phospholipid fractions and their concentrations to gestational age and the development of RDS in infants of healthy mothers, mothers with diabetes mellitus and mothers with Rh sensitization. The following is a report of these studies.

MATERIALS

Amniotic fluid was obtained via amniocentesis from mothers delivering at the Kvinneklinikken who were diabetic, sensitized Rh negative, had pre-eclampsia or received care elsewhere in Norway. The fluid was centrifuged and refrigerated at −20°C until analyzed. Fluids contaminated with blood or meconium were not used. In a number of cases it was possible to have serial samples of fluid during the last trimester of pregnancy.

A diagnosis of RDS required the presence of symptoms of respiratory distress (tachypnoea, grunting, retracting) beginning in the first 6 hours of life presenting for more than 24 hours and of sufficient severity to require supplemental oxygen for more than 24 hours. In 3 infants, the diagnosis was verified by autopsy. One infant with prolonged complicated delivery and possible cerebral injury is not included in the category of RDS.

Since the mothers having amniocentesis were a selected group and did not include all cases with a single diagnosis from any one institution, the data cannot be used to determine frequency of disease or incidence [illegible] population.

METHODS

The lipids were extracted using Bligh and Dyer's proportions (10) as follows: 4 ml of amniotic fluid was mixed with 15 ml of chloroform methanol (1 2) and shaken for a few minutes in a separatory funnel giving the proportions of 1 2 0.8 of chloroform/methanol/water (C M W) respectively before dilution. The mixture was then filtered through a sintered glass filter using positive pressure from above with purified nitrogen. Five ml of chloroform was washed through the filter to remove any lipid adhering to the protein. Five ml of distilled water was then added to the mixture giving the final proportions of 2 2 1.8 of C M W. Satisfactory separation was always achieved after the final shaking of the mixture. This procedure ensures minimum non-lipid conta

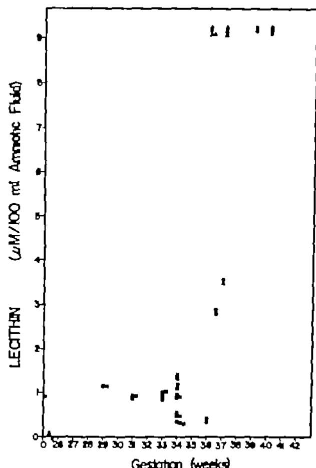


Fig 1 Lecithin concentration in amniotic fluid related to gestational age. Δ – Diabetic, □ – Rh neg, – Other

mination of the chloroform phase The lower lipid containing chloroform layer was removed and concentrated in a rotary evaporator Th concentrated lipid was transferred to a small vial using three washings with chloroform and dried nder a stream of purified nitrogen in water bath at 50°C.

The sample was dissolved in 0.2 ml chloroform and spotted in duplicate on activated TLC. The chromatography plates were prepared using silica gel-H without binder and spread to 0 5 mm thickness. After tank development in chloroform, methanol, water (65 25 4) for approximately 45 min the lipid fractions were lightly stained with iodine vapors in a similar tank. Fractions were identified using standards. The chromatogram was divided into six fractions and each was scraped from the plate into glass tubes and digested for 1 hour at 190°C with 0.4 ml perchloric acid. Distilled water (3.. ml) was added to the digestion tubes, vortemixed nd centrifuged at 2 000 rpm for 10 min. Three ml of clear supernatant (5/6 of the total sample) was aspirated with a 3 ml volumetric pipette. Reagent blanks and phosphat standards were treated similarly Phosphate was determined by adding 0.3 ml 2.5% ammonium molybdate with 3% H_2SO and adding 0.3 ml of freshly made absorbic acid. After light mixing, the tubes were boiled in w ter bath for 7 min and read photometrically at 830 μ The values of the lipid fractions are calculated as micromoles of lipid in 100 ml of amniotic fluid. Testing the efficiency of direct silica gel digestion was done as follows: 5 λ of concentrated egg yolk extract was pipetted directly into test tubes in duplicate and the same amounts were spotted and developed on TLC. After dividing spots and digesting as outlined, the compiled lipid content of all spots should equal the amount placed directly into tubes. Fifteen determination gave recovery of 95-105%. The six fractions analysed from the origin were:

1) lysolecithin, 2) sphingomyelin, 3) lecithin, 4) not yet identified, phosphatidylethanolamine.

RESULTS

There were 118 samples of amniotic fluid obtained from 72 mothers. Of these, 82 samples were from 40 Rh sensitized mothers, 19 samples were from 15 diabetic patients, and 17 samples were from healthy mothers or from mothers who had pre-eclampsia.

The concentrations of lecithin related to gestational age are presented in Fig. 1 Prior to 33¹/₂

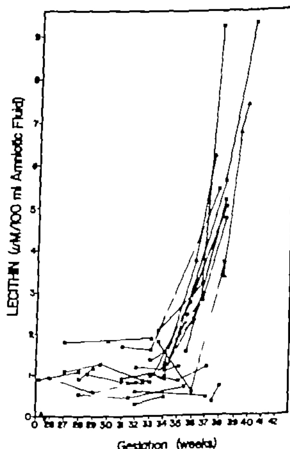


Fig 2. Lecithin concentration related t gestational age in sequential samples from mothers. Δ – Diabetic □ – Rh neg.

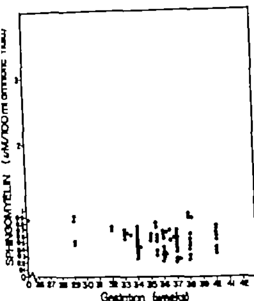


Fig 3 Sphingomyelin concentration related to gestational age

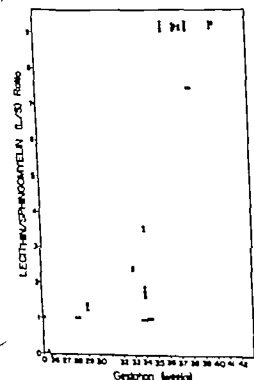


Fig 4. Lecithin/sphingomyelin ratios related to gestational age Δ Diabetic, □ = Rh neg. Other

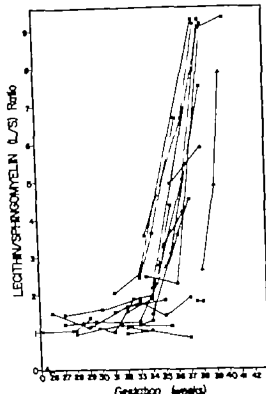


Fig 5. L/S ratios related to gestational age in sequential samples Δ Diabetic, □ = Rh neg

weeks all samples contained <2.0 μg/100 ml of lecithin. Concentrations >4 μM/100 ml were first noted at 35 weeks and were frequently found after 36 weeks. Between 36 and 38 weeks gestation, there were eight fluid samples containing less than 1 μM/100 ml. All the samples obtained prior to 34 weeks gestation were from Rh negative mothers. After 34 weeks, the samples from diabetic mothers generally had higher concentrations of lecithin than those from Rh sensitized mothers, although there was a wide range of values in both groups.

The concentrations of lecithin in the amniotic fluid from 21 mothers ha ing 65 amniocenteses are presented in Fig. 2. Although a marked increase in the lecithin concentrations occurs in most patients between 34 and 38 weeks, 5 patients maintained low concentrations beyond 35 weeks gestation (<1.5 μM/100 ml). One patient had a drop in lecithin concentration between 34 and 36 weeks followed by marked increase again t 38 weeks gestation. One patient had a lecithin

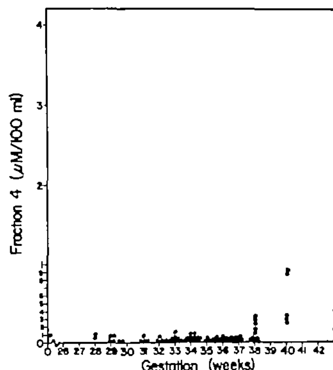


Fig 6 Fraction 4 concentrations related to gestational age

concentration <1 μM/100 ml at 38 weeks gestation After 38 weeks all patients had lecithin concentrations over 1 μM/100 ml.

The concentrations of lysolecithin varied between 0 and 0 29 μM/100 ml with a mean of

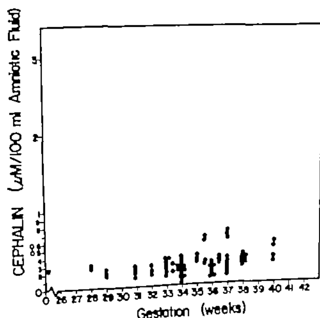


Fig 7 Phosphatidylethanolamine concentrations related to gestational age.

0 10 μM/100 ml. The concentration has no relationship to gestation. Although most samples with lysolecithin concentrations >0.. μM/100 ml had high total phospholipid concentrations, a number of samples with high total phospholipid content had low (<0.10 μM/100 ml) lysolecithin concentrations.

The concentrations of sphingomyelin at different gestational ages are presented in Fig 3 Only 8 of 118 samples had concentrations over 1 0 μM/100 ml There was little change with gestational age with perhaps a slight decrease at 32 weeks.

The ratios of lecithin concentration to sphingomyelin concentrations are presented in Fig. 4. The distribution of gestational age follows the pattern for lecithin concentrations shown in Fig 1 Between 37 and 40 weeks a few patients with lecithin concentrations between 1.5 and 3.0 μM/100 ml had high L/S ratios. Only 2 patients with lecithin concentrations <1 0 μM/100 ml had L/S ratios over 2.0. The patterns of L/S ratios for patients having multiple amniocenteses (Fig 5) are likewise similar to the pattern shown in Fig. 2. Between 33 and 36 weeks nearly all patients show a sharp rise in the ratio of lecithin to sphingomyelin.

Fraction 4 also increased in concentration with gestational age beginning at 35–36 weeks (Fig 6) Prior to 35 weeks gestation the concentration was less than 0 2 μM/100 ml. On the chromatogram one to four bands could be distinguished. No attempts were made to identify the bands or isolate them separately

The concentrations of phosphatidylethanolamine by gestational age are presented in Fig. 7 The concentration remains nearly constant until 35–36 weeks gestation with a small increase between 36 and 40 weeks. Only three samples had more than 1 0 μM/100 ml.

The solvent front fraction containing the phosphotidic acid had from 0 to 0.41 μM/100 ml phospholipid. There was no relationship to gestational age total lipid concentration or concentration of the phospholipids.

The total phospholipid concentration in amniotic fluid, giving the six fractions at different gestational ages, are presented in Fig. 8 The observed increase in total phospholipid is largely lecithin with some increase in fractions 4 and 5

The relationship of lecithin concentrations to

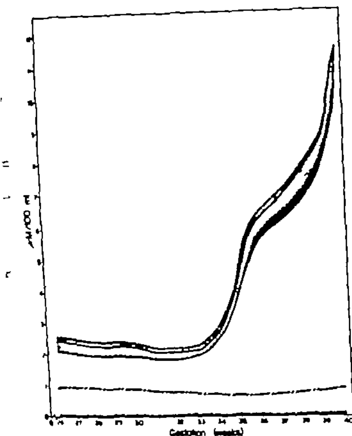


Fig. 8. Total phospholipid concentrations and compiled averages of each fraction from starting point to solvent front related to gestational age.

——— = lysolecithin,
——— = sphingomyelin,
——— = lecithin,
– – – – Fraction 4
——— = phosphatidylethanolamine,
—— —— solvent front.

the development of RDS at different gestational ages is presented in Fig. 9. These samples were obtained within one week of delivery and the results are correlated with the presence or absence of RDS in the newborn. All the infants (twelve) who developed RDS had lecithin concentrations less than 1.2 μM/100 ml. The infants with no respiratory distress all had concentrations > 1.3 μM/100 ml. In this series of 55 samples there were only two samples obtained prior to 34 weeks in which delivery occurred within 1 week of the amniocentesis.

The relationship of the L/S ratio to the development of RDS is presented in Fig. 10. As with the lecithin concentration those infants who developed RDS has low ratios (< 0) while the infants without distress had ratios > 5.

Seventeen patients delivered from 1.5 to 7 weeks after the last sample was obtained. These are presented in Table I. While some of these infants had distress and others were healthy it

Table I. *Lecithin and sphingomyelin concentrations not closely associated with delivery*

Name	Maturity Fluid	Maturity Child	Lecithin Conc.	Sphing. Conc.	L/S	Child's cond.
K. S	33	34.5	1 92	1 04	1 84	Normal
K. F	34	35 5	2.75	0 40	6 9	Normal
T B.	35 5	37	4 68	0 52	9 0	Normal
F O.	34 5	38	2 93	0 70	4 2	Normal
O F	33	35	0 94	0 67	1 4	RDS
L. T	34	36	0 35	0 35	1 0	RDS
M W	37	39	1 29	0.71	1 80	Normal
R H.	35	37.5	1 19	0 56	2.12	Normal
K. L	36	38.5	0 37	0 29	1 28	RDS
R. H	34	37	0 38	0 64	0 59	Normal
A F	29	34	1 16	0 86	1 36	Normal
A M.	34	38	0 48	0 48	1 0	RDS
A L. K	29	34	0 76	0 54	1 41	RDS
M E	30 5	36	0 76	0 72	1 05	Normal
J T	29	36	1 17	0 92	1 27	RDS
E. T	29 5	35 5	0 83	0 80	1.04	RDS
A U T	28	35	1 09	0 99	1 10	RDS

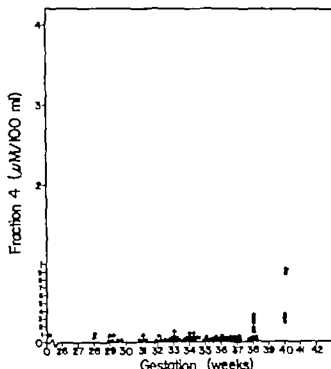


Fig 6 Fraction 4 concentrations related to gestational age.

concentration <1 µM/100 ml at 38 weeks gestation. After 38 weeks all patients had lecithin concentrations over 1 µM/100 ml.

The concentrations of lysolecithin varied between 0 and 0 29 µM/100 ml with a mean of 0.10 µM/100 ml. The concentration has no relationship to gestation. Although most samples with lysolecithin concentrations >0.2 µM/100 ml had high total phospholipid concentrations, a number of samples with high total phospholipid content had low (<0 10 µM/100 ml) lysolecithin concentrations.

The concentrations of sphingomyelin at different gestational ages are presented in Fig 3 Only 8 of 118 samples had concentrations over 1 0 µM/100 ml. There was little change with gestational age with perhaps a slight decrease at 32 weeks.

The ratios of lecithin concentration to sphingomyelin concentrations are presented in Fig 4 The distribution of gestational age follows the pattern for lecithin concentrations shown in Fig 1 Between 37 and 40 weeks a few patients with lecithin concentrations between 1.5 and 3 0 µM/100 ml had high L/S ratios. Only 2 patients with lecithin concentrations <1 0 µM/100 ml had L/S ratios over 2.0. The patterns of L/S ratios for patients having multiple amniocenteses (Fig 5) are likewise similar to the pattern shown in Fig. 2 Between 33 and 36 weeks nearly all patients show a sharp rise in the ratio of lecithin to sphingomyelin.

Fraction 4 also increased in concentration with gestational age, beginning at 35–36 weeks (Fig. 6) Prior to 35 weeks gestation the concentration was less than 0.2 µM/100 ml. On the chromatogram one to four bands could be distinguished. No attempts were made to identify the bands or isolate them separately

The concentrations of phosphatidylethanolamine by gestational age are presented in Fig. 7 The concentration remains nearly constant until 35–36 weeks gestation with a small increase between 36 and 40 weeks. Only three samples had more than 1 0 µM/100 ml

The solvent front fraction containing the phosphatidic acid had from 0 to 0 41 µM/100 ml phospholipid. There was no relationship to gestational age total lipid concentration or concentration of the phospholipids.

The total phospholipid concentration in amniotic fluid, giving the six fractions at different gestational ages, are presented in Fig. 8. The observed increase in total phospholipid is largely lecithin with some increase in fractions 4 and 5.

The relationship of lecithin concentrations to

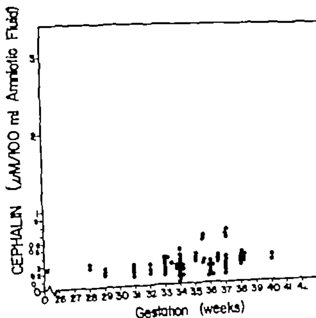


Fig 7 Phosphatidylethanolamine concentrations related to gestational age.

Sphingomyelin shows some individual variation, with concentrations usually $<1.0\ \mu M/100$ ml, with little relation to gestational age and none to maternal disease or fetal outcome.

Fraction 4 starts to rise from 35–36 weeks in those infants where maturation proceeds normally. Very low or unrecordable concentrations were found in patients where lecithin levels were generally low. No relationship was found to maternal disease.

Cephalin (Fraction 5) was always present and started to rise between 35 to 36 weeks gestation in a pattern similar to Fraction 4. No other correlation could be made.

In relationship to the development of RDS to lecithin concentrations $<1.3\ \mu M/100$ ml seemed to be critical. Since the relationship of sphingomyelin to pulmonary surfactant quality and quantity is not clear, it may be that its concentration can be used as a dilutional factor and therefore be of use in assessing pulmonary maturity. The 12 patients with lecithin concentration $<1.2\ \mu M/100$ ml all had L/S ratios <2.0 and this strongly supports Gluck's statement. A closer correlation of lecithin concentration and L/S ratio to the severity of the distress has not been done in this report, since the outcome is influenced by many other factors in addition to the alveolar surfactant lining. However, very low levels of lecithin and L/S ratios tended to be associated with severe distress.

With regard to maternal disease, our findings of delayed pulmonary maturation in some Rh sensitized mothers may indicate that this delay is related to the severity of the erythroblastosis.

Similar findings may have been made by C. R. Whitfield et al. (10). Pulmonary maturation proceeded normally in all diabetic patients except one. This may be related to their long in-patient care before delivery.

The concentration of lecithin and the L/S ratio appear to be good indicators of fetal pulmonary maturity after 34 weeks gestation. There are insufficient numbers of samples obtained near the time of delivery and prior to 34 weeks to draw any conclusion. Samples near delivery from a fairly large group of mothers whose babies are born prior to 34 weeks gestation is needed. In the interval from 28 to 33.5 weeks, 32 phospholipid determinations were made. Eight of these had lecithin levels $>1.3\ \mu M/100$ ml and six in the group of eight also had an L/S ratio >2. The other two had sphingomyelin concentrations $>1.0\ \mu M/100$ ml. It remains to be seen whether the values in this age group are of significance.

Reasonably accurate predictions regarding fetal pulmonary function could be made when the amniotic fluid phospholipid levels are high, but for such predictions to be made with a high degree of confidence, the method for extraction and the phospholipid determinations must be carefully performed. Since part of the object of this study was to determine the total phospholipid pattern in the last trimester, acetone precipitation of the lipid extract was not done. Our experiences with that procedure in order to purify surface active lecithin has to date not shown any advantage over the method presently described.

CONCLUSION

The findings of this study show that lecithin concentrations in amniotic fluid begin to increase sharply from 34 weeks in infants where pulmonary maturation proceeds normally. Increase in L/S ratios are related to increase in lecithin. Respiratory distress syndrome has been shown to be associated with lecithin concentrations $<1.3\ \mu M/100$ ml and an L/S ratio <2.0. Delayed pulmonary maturation may occur in infants of diabetic mothers and erythroblastic infants. It is suggested that this may be related to the severity of the condition.

ACKNOWLEDGEMENTS

We are indebted to Professor M. Seip, Department of Pediatrics, Professor Bjøro, Department of Obstetrics and Gynecology, Dr Per Finne, Dr H. Jensen (Aker Sykehus), the Hartford Foundation, and Norske K. [illegible] Sanitetsforening for making this study possible.

REFERENCES

1. Bhagwanani, S. G. et al. Prediction of neonatal respiratory distress by extraction of amniotic fluid lecithin. Lancet 1: 77, 1972.
2. Bligh & Dyer: A rapid method of total lipid extraction and purification. Can J Biochem 37: 911, 1959.
3. Gluck, L. et al. Diagnosis of the respiratory distress syndrome by amniocentesis. Am J Obstet Gynecol 109: 440, 1971.
4. Gluck, K. Procedure for the intrauterine assessment of pulmonary maturity by the phospholipids in am-

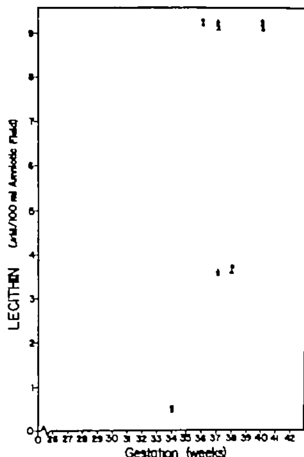


Fig 9 Lecithin concentration related to gestational age and fetal outcome. Δ = Diabetic, □ = Rh neg, = Other Solid characters represent patients who delivered babies with RDS.

was not possible to correlate the development of RDS to the fluid lipid composition when the fluid was not closely related to the time of delivery Twelve of these infants had lecithin concentrations < 1 3 µM and L/S ratios < 2.0 Eight developed RDS after delivery and four were healthy as were the other five.

There was no difference between infants of diabetic mothers and infants of Rh sensitized mothers in the relationship of lecithin concentrations and L/S ratio to the development of RDS. The one infant of a diabetic mother who developed RDS has a lecithin concentration of 1 12 µM/100 ml and an L/S ratio of 1 7 at 36 weeks gestation.

Phospholipid fraction 4 showed 0 in all samples associated with RDS except in one patient where 0 07 and 0 08 µM/100 ml was found at 25 and 28 weeks gestation respectively Cephalin levels showed no particular relationship to RDS.

DISCUSSION

The studies of amniotic fluid phospholipid concentrations have hitherto mainly been concerned with lecithin and sphingomyelin levels in relationship to gestational and in relationship to the respiratory distress syndrome. Nelson (9) previously reported the phospholipid concentration in 30 patients from 37–40 weeks gestation. A quantitative study of all six phospholipid fractions throughout the last trimester in relation to gestational age, maternal disease and fetal outcome has to our knowledge not previously been reported.

Our findings show that the lecithin fraction start to rise sharply from 34 weeks, and apparently associated with normal pulmonary maturation This is in agreement with the findings of Graven (7) and Gluck (3). Lysolecithin and the solvent front shows uniformly low values throughout the last trimester and shows no relationship to either gestation, maternal disease or fetal outcome.

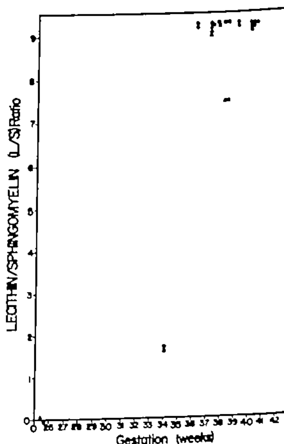


Fig 10 L/S ratios related to gestational age and fetal outcome Δ = Diabetic □ = Rh neg, = Other Solid characters represent patients who delivered babies with RDS.

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FIBRINOLYTIC INHIBITORS IN HUMAN RETROPLACENTAL BLOOD

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Abstract. Fibrinolytic inhibitors of plasminogen activation (urokinase inhibitors) and of plasmin (alpha$_2$-macroglobulin, antiplasmin and total antitrypsin activity) were measured in retroplacental blood and compared with that in placental extract and maternal venous blood obtained at 40 deliveries. In placental extract the concentration of inhibitors of plasminogen activation was extremely high. In contrast, it was found to be normal in retroplacental blood and close to the value found for maternal venous blood. It therefore seems unlikely that inhibitors from the placenta enter the bloodstream and contribute to the reduction of the fibrinolytic activity in the maternal circulation during pregnancy.

At the end of pregnancy the fibrinolytic activity of the blood is substantially reduced (2, 3, 5, 11, 15, 19). During labour it is somewhat elevated, but still low compared with that in non-pregnant women (22). After delivery it rapidly returns to normal levels (2, 4, 20, 23). Recently it has been shown that the depression of the fibrinolytic activity during pregnancy is dependent on the presence of the placenta and not of the fetus (24).

It is well known that the placenta contains large amounts of urokinase inhibitors (1, 13). These inhibitors might be *responsible* for the changes in the fibrinolytic activity. However, in the blood they have been found to be increased (6, 14, 18), normal or even decreased (3, 7, 12, 15). It is thus unsettled whether the inhibitors demonstrated in the placenta also enter the bloodstream.

As far as we know, the retroplacental blood has never before been investigated for inhibitors of fibrinolysis. This paper concerns fibrinolytic inhibitors in retroplacental blood. These inhibitors were compared with those in extracts of placenta and of maternal venous blood.

MATERIAL AND METHODS

Retroplacental blood was obtained at 40 normal deliveries. Blood samples were also drawn from an antecubital vein shortly before delivery. Serum was prepared as described previously (16).

Placental extract was prepared according to Abildgaard & Ungrudd (1).

Inhibitors of plasminogen activation by urokinase (urokinase inhibitors). Clot method (16). Normal range 60–140%.

Antiplasmin. Caseinolytic method by Shamash & Rimon (17), as modified by Eklund et al. (8). Normal range 80–120%.

Alpha$_2$-macroglobulin. Esterolytic method (10). Normal range 50–150%.

Total antitrypsin activity (TAT). Esterolytic method (9). Normal range 70–150%.

The results are expressed relative to the content of a normal standard consisting of pooled serum from 20 apparently healthy persons. For comparison between the amount of inhibitors found in extracts of the placenta with that found in venous and retroplacental blood, the total protein was determined and the concentration of the inhibitors was corrected for 7 g/100 ml protein.

RESULTS

The values found for the inhibitors investigated were approximately equal in the retroplacental blood and venous blood but differed markedly from those in extracts of the placenta (Table I).

Extracts of the placenta contained an extremely high concentration of inhibitors of urokinase-induced plasminogen activation. The values found for alpha$_2$-macroglobulin, total antitrypsin and antiplasmin activity were low (Table I).

DISCUSSION

The inhibition of urokinase-induced plasminogen activation in a given patient was thus near that

niotic fluid. Newsletter Univ of Cal, San Diego. Aug. 1 1971

5. Graven, S. N & Meisenheimer H R Respiratory distress syndrome and the high risk mother Am J Dis Child *109* 489 1965
6. Graven S. N et al. The respiratory distress syndrome. Am J Obstet Gynecol 99 969 1969
7. Graven S. N Incorporation of choline, ethanolamine, serine and S-adenosylmethionine into phospholipids of fetal primate lungs. Proc Soc Ped Res, Atlantic City N J., 1968
8. Nelson, G H Amniotic fluid phospholipid patterns in normal and abnormal pregnancies. Am J Obstet Gynecol *105* 1072, 1969
9. Nelson, G H. et al., Thin-layer chromatography of placental phospholipids. Am J Obstet Gynecol *11* 4, 1972.
10. Whitfield, C. R. et al., Amniotic fluid lecithin Sphingomyelin ratio and fetal lung development. Br Med J 2. 85–86 April 8, 1972.

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7. Correll, J. T. & Sjoerdsma, A.: Inhibition of human fibrinolytic system by normal and pathologic sera. Proc Soc Exp Biol (NY) *111*: 274, 1962.
8. Ekelund, H., Hedner, U. & Nilsson, I. M.: Fibrinolysis in newborns. Acta Paediat Scand *59*: 33, 1970.
9. Eriksson, S.: Studies in α_1-antitrypsin deficiency. Acta Med Scand Suppl 432, 1965.
10. Ganrot, P. O.: Determination of α_2-macroglobulin as trypsin-protein esterase. Clin Chim Acta *14*: 493, 1966.
11. Gillman, T., Naidoo, S. S. & Hathorn, M.: Plasma fibrinogen activity in pregnancy. Lancet *II*: 70, 1959.
12. Hedner, U. & Astedt, B.: Studies on fibrinolytic inhibitors during pregnancy. Acta Obstet Gynecol Scand *50*: 91, 1971.
13. Kawano, T., Morimoto, K. & Uemura, Y.: Urokinase inhibitor in human placenta. Nature *217*: 253, 1968.
14. Laurinen, O. S.: The fibrinolytic enzyme system of human blood plasma in pregnancy. Scand J Clin Lab Invest *23*: 191, 1969.
15. Nilsson, I. M. & Kullander, S.: Coagulation and fibrinolytic studies during pregnancy. Acta Obstet Gynecol Scand *46*: 273, 1967.
16. Paraskevas, M., Nilsson, I. M. & Martinsson, G.: A method for determining serum inhibitors of plasminogen activation. Scand J Clin Lab Invest *14*: 138, 1962.
17. Shamash, Y. & Rimon, A.: The plasmin inhibitors of plasma. I. A method for their estimation. Thromb Diath Haemorrh (Stuttg.) *12*: 119, 1964.
18. Shaper, A. G., Kear, J., Macintosh, D. M., Kyobe, J. & Njama, D.: The platelet count, platelet adhesiveness and aggregation and the mechanism of fibrinolytic inhibition in pregnancy and the puerperium. J Obstet Gynaecol Br Commw *75*: 433, 1968.
19. Shaper, A. G., Macintosh, D. M., Evans, C. M. & Kyobe, J.: Fibrinolysis and plasminogen levels in pregnancy and the puerperium. Lancet 2: 706, 1965.
20. Shaper, A. G., Macintosh, D. M. & Kyobe, J.: Fibrinolytic activity in pregnancy, during parturition, and in the puerperium. Lancet *II*: 874, 1966.
21. Astedt, B.: On fibrinolysis. A. In pregnancy, labour, puerperium and during treatment with sex hormones. B. In human ontogenesis and in human organ cultures. Acta Obstet Gynecol Scand, Suppl. *18*, 1972.
22. — Fibrinolytic activity during labour. Acta Obstet Gynecol Scand *51*: 171, 1972.
23. — Fibrinolytic activity of veins in the puerperium. Acta Obstet Gynecol Scand *51*: 325, 1972.
24. — Demonstration of significance of placenta in depression of fibrinolytic activity during pregnancy. J Obstet Gynaecol Br Commw *79*: 205, 1972.
25. Astedt, B., Isacson, S., Nilsson, I. M. & Pandolfi, M.: Fibrinolytic activity of veins during pregnancy. Acta Obstet Gynecol Scand *49*: 171, 1970.
26. Astedt, B., Pandolfi, M. & Nilsson, I. M.: Inhibitory effect of placenta on plasminogen activation in human organ culture. Proc Soc Exp Biol Med *139*: 1421, 1972.

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Table I *Mean values of fibrinolytic inhibitors in placental extract retroplacental blood and maternal venous blood obtained at 40 deliveries (expressed relative to pooled serum from normals and corrected for 7 g/100 ml protein)*

	Placental extract	Retro-placental blood	Venous blood	Normal range in non pregnant normals
Urokinase inhibitors,	4 307	102	76	60–140
Alpha$_2$-macroglobulin, %	61	116	135	80–150
Antiplasmin %	11	180	199	80–120
Antitrypsin, %	Traces	120	138	70–140

of venous blood from the same patient, and did not exceed that in serum from non-pregnant women.

In extracts of the placenta the concentration of urokinase induced plasminogen activation was extremely high, corroborating the figures given by Kawano et al (13) and Abildgaard & Uszynski (1) The small amounts of alpha$_2$-macroglobulin and alpha$_1$-antitrypsin and antiplasmin activity in the placental extract, which have hitherto passed unnoticed might be due to contaminating blood.

The cause of the marked reduction of the fibrinolytic activity at term and its rapid return after delivery is still debatable Åstedt et al. (25) found a low content of fibrinolytic activators in the vessel wall during pregnancy as well as a successively decreasing response of the fibrinolytic activity to venous occlusion Åstedt (24) also assessed the local response of the fibrinolytic activity to venous occlusion 1 hour after parturition in women in whom the placenta had been retained and compared it with that in a series where delivery of both the child and the placenta had been normal The local response of the fibrinolytic activity was still low in women with retention of the placenta, but almost normal in women in whom the delivery of both child and placenta had been normal

This finding shows that the depression of the fibrinolytic activity of the blood during pregnancy is dependent on the presence of the placenta and not on that of the fetus.—As sex-hormones are known to influence fibrinolytic activity (21) the secretion of steroid hormones by the placenta might directly or indirectly act upon the synthesis and release of fibrinolytic acitvators in the vessel wall.

But the depressive effect of the placenta might also be ascribed to its large content of inhibitors of urokinase-induced plasminogen activation (1, 13) Åstedt et al (26) in combined organ culture studies have demonstrated that the inhibitors released from placenta explants inhibit not only the activity of urokinase, but also the fibrinolytic activators released from vessel explants A decisive question is now whether the placental inhibitors enter the maternal blood-stream. As mentioned above, opinions differ concerning the urokinase-inhibiting effect of pregnant blood

In the present investigation, the concentration of inhibitors of urokinase-induced plasminogen activation in retroplacental blood was found to be 102% of that in serum from normal non-pregnant women. This normal value contrasts with the extremely high concentration of urokinase inhibitors found in the placental extract. It therefore seems unlikely that urokinase inhibitors from the placenta enter the maternal bloodstream and reduce fibrinolytic activity in the maternal circulation. The physiological purpose of the placental urokinase inhibitors is probably to secure more effective haemostasis in this organ

ACKNOWLEDGEMENT

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REFERENCES

1 Abildgaard, U & Uszynski, M Separation and characterization of two fibrinolytic inhibitors from human placenta Thromb Diath Haemorrh 25 590, 1971
2. Biezenski, J J & Moore, H C Fibrinolysis in normal pregnancy J Clin Path 11 306, 1958
3 Bonnar J McNicol, G P & Douglas, A S Fibrinolytic enzyme system and pregnancy B Med J 3 387 1969
4 Bonnar J McNicol, G P & Douglas, A S Coagulation and fibrinolytic mechanisms during and after childbirth. Br Med J 11 200, 1970
during pregnancy Amer J Obstet Gynecol 94 14 1966.
5 Brakman, P The fibrinolytic system in human blood
6 Brakman, P & Astrup, T Selective inhibition in human pregnancy blood of urokinase induced fibrinolysis. Scand J Clin Lab Invest 15 603, 1963

Acta Obstet Gynec Scand 53 231–233 1974

DETECTION OF FETAL HEART ACTIVITY DURING EARLY PREGNANCY BY COMBINED B-SCAN AND DOPPLER EXAMINATION A NEW APPLICATION

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Abstract 99 pregnant women, 5 to 11 weeks, were examined by combined ultrasonic B-scan and Doppler method to detect fetal heart activity The fetus was localized accurately by B-scanning and the heart activity was detected through the mother's abdominal wall with 100% certainty at eight weeks of pregnancy (menstrual age). The earliest fetal heart activity detected was in pregnancy which dated 44 days from the last menstrual period Positive findings were recorded on tape and photographed from the oscilloscope screen.

It is often difficult to determine whether the pregnancy will continue in women with bleeding during early pregnancy Gynecological examination and immunological pregnancy tests on the urine do not give an immediate answer to the question of whether the fetus is living or dead. On the other hand, an accurate prognosis can be given immediately if fetal heart activity or absence thereof can be determined reliably Ultrasonic examination based on the Doppler effect has now been used for nearly a decade for detection of fetal heart activity It has proved to be reliable method for detecting heart activity through the abdominal wall from the 12th week of pregnancy onwards (1 2, 5) By vaginal Doppler technique fetal heart activity has been observed at 8 weeks, and 100% certainty has been achieved in examinations at 10 weeks of pregnancy (3) A vaginal examination is, however uncomfortable for the patient and difficult for the doctor making the examination. Also it may increase the risk of abortion in cases of threatened abortion This study deals with a method of detecting fetal heart activity through the abdominal wall with the help of combined Doppler and B-scan techniques.

METHOD AND MATERIAL

In this research 5 MHz ultrasonic Doppler instrument (Picker EUD 1 B) was used, with a maximum source intensity of 20 mW/cm² For ease of precise handling, plastic handle was attached to the normal planar probe of this instrument The surface diameter of the probe was 20 mm. B-scanning was made with Kretztechnik B-scan equipment (4100 MGB) and 2 MHz probe. For both examinations olive oil was used as contact medium.

The examination of the patient was started with B-scanning. Efforts were made to obtain on the storage tube clear picture of the uterus and the gestation sac Echoes, if any from inside the gestation sac were localized (Fig 1) The ultrasonic beam was pointed directly at the fetus, and the position and tilt of the probe were carefully noted. Thereafter the B-scan probe was removed from the patient's skin and the Doppler probe was placed on exactly the same spot and at the same angle. By minute tilting of the probe in different directions but without changing its place, fetal heart sounds were usually heard, in the case of normal pregnancy after searching for 1–2 minutes The sounding was made with earphones A tape recorder and an oscilloscope (Tektronix type 563) were connected to the Doppler instrument By pressing foot switch the heart sounds were transposed to the tape recorder and the oscilloscope. Every positive finding was recorded on tape and on photograph of the oscilloscope record (Fig 2) If no heart sounds could be found in about 3 minutes the finding was recorded as negative.

In the course of this investigation 99 pregnant women, whose menstrual cycles had been regular and whose starting day of the last menstruation was known with certainty were examined Their ages of pregnancy ranged from 5 to 11 weeks amenorrhoea 84 of the women had clinically normal pregnancies The remaining 15 were admitted to hospital with symptoms of threatened abortion. The later development of the pregnancy was checked in each case

RESULTS

The results are presented in Table 1. The table indicates the number of cases, the distribution by

heart activity was detected was 44 days from the beginning of the last menstruation.

The investigation indicates that with the help of B-scan it is possible to detect fetal heart activity by Doppler technique through the abdominal wall of a considerably earlier stage of pregnancy than has been achieved in earlier studies (1, 2, 5). Not even the vaginal Doppler technique (3) confirms with such certainty and at such an early stage of pregnancy the fact that the fetus is living as does the combined B-scan and Doppler examination.

The fact that previously the Doppler method has been unreliable for detecting fetal heart activity through the abdominal wall before the 12th week may have been caused by the difficulties in localizing the fetus. Detecting heart activity during the early weeks of pregnancy involves, besides knowledge, of the location of the gestation sac, also the location of the fetus within the sac. The rod-like shape of the Doppler probe is most advantageous in facilitating directing of the ultrasonic beam of the Doppler equipment upon the fetus detected by B-scanning.

In the early stage of the research a number of patients were examined both by 2 MHz and 5 MHz Doppler equipment. By 5 MHz Doppler equipment it seemed to be easier to detect the heart activity of the fetus before 10 weeks. The observation coincides with that of Jouppila (3). Although comparative examination between equipment was not made the 2 MHz equipment was abandoned. Obesity of the patient or retroversion of the uterus were no obstacle to a correct diagnosis. A full bladder was a prerequisite for a successful examination. On the other hand, an over-stretched bladder was inconvenient as it pressed upon the uterus thus making it difficult to obtain a distinct B-scan and detect the fetal echoes.

The combined B-scan and Doppler method compares well with other ultrasonic methods employed in detecting fetal heart activity. Kratochwil & Eisenhut (4) detected fetal heart activity by vaginal A-scanning in a six-week pregnancy (44 days of amenorrhoea) and achieved 100% certainty from 9 weeks onwards. However the method has the same disadvantages as the vaginal Doppler technique. Robinson (6), using the new Diasonograph NE 4102 of Nuclear Enterprises Ltd. and the combined B and TP techniques, detected fetal heart activity in a pregnancy of 48 days of amenorrhoea. In this study covering 56 cases, neither false positive nor false negative findings were obtained. According to Robinson (6, 7) this result is possible only if a readily controllable high-grade sensitive ultrasonic equipment is available. As in ultrasonic diagnostics generally here too the individual skill of the examiner naturally plays an important part.

The possibility of detecting fetal heart activity or its absence and of quickly finding out the reason for bleeding in early pregnancy shortens the patient's time of treatment and reduces the risk of infection.

REFERENCES

1. Bernstine, R. L. & Callagan, D. A. Ultrasonic doppler inspection of the fetal heart. Amer J Obstet Gynec *95*: 1001, 1966.
2. Johnson, W. L., Stegall, H. F., Lein, J. N. & Rushmer, R. F. Detection of fetal life in early pregnancy with an ultrasonic Doppler flowmeter. Obstet Gynec *26*: 305, 1965.
3. Jouppila, P. Ultrasound in the diagnosis of early pregnancy and its complications. Acta Obstet Gynec Scand *50*: Suppl. *15*, 1971.
4. Kratochwil, A. & Eisenhut, L. Der früheste Nachweis der fetalen Herzaktion durch Ultraschall. Geburtsh Frauenheilk *27*: 176, 1967.
5. Kosh, K. B. & Embrey, M. P. Experiences with an ultrasonic foetal pulse detector. Brit Med J *1*: 433, 1968.
6. Robinson, H. P. Detection of fetal heart movement in first trimester of pregnancy using pulsed ultrasound. Brit Med J *4*: 466, 1972.
7. Robinson, H. P. Personal communications 1973.

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Fig 1 Longitudinal section of the uterus of an eight week pregnancy The fetal echoes (*F*) are clearly distinguished within the gestation sac. *Bl* = bladder *F* = fetus, *GS* = gestation sac, *V* = vagina.

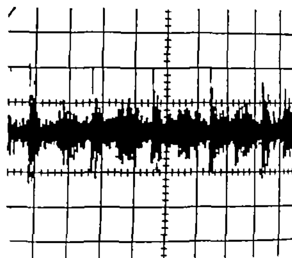


Fig 2. Doppler signal of the heart of a seven-week fetus photographed from oscilloscope screen. The biphasic character of the heart activity is clearly indicated Sweep speed 0.. sec/cm.

gestation period and the weekly Doppler findings. Fetal heart activity was detected in 72 cases. No false positive findings were obtained. Fetal heart activity was detected in two cases at six weeks gestation (44 days and 48 days from the start of the last menstruation). At seven weeks gestation a correct positive finding was obtained in ten and a false negative finding in six cases. In pregnancy lasting 8 weeks or more no false negative findings were obtained

Following the course of the pregnancy confirmed the correct negative finding in seven cases. Three of these, who had a negative urine immunological pregnancy test (Pregnosticon®) and bleeding, underwent uterine evacuation and curettage inside 9 days after the ultrasonic examination. The operative findings suggested a pregnancy aborted a long time before and the pathologic-anatomical diagnosis was in each case "Retained products of conception" Three patients aborted spontaneously one on the day following the ultrasonic examination and two within a week after the examination. The pathologic-anatomical diagnosis was in each case Pathological ovum One patient delivered a very macerated fetus, smaller than expected for the gestation period, 2 $^1/_2$ weeks after the ultrasonic examination.

DISCUSSION

In this research, the correct diagnosis of the fetal heart activity or its absence was made in 79 cases out of a total of 99 patients, whose duration of pregnancy ranged from 5 to 11 weeks. When the pregnancy had reached eight weeks 100% diagnostic accuracy was obtained. No false positives occurred. False negative findings were obtained in 37 5% of the seven week examinations and in 60 0% of the six week examinations. The earliest stage of pregnancy at which the fetal

Table I *Weekly results of the observations on fetal heart activity made by the combined B-scan and Doppler method*

Findings	Weeks of pregnancy 5	6	7	8	9	10	11	Total
Correctly positive	—	2	10	19	15	15	11	72
False positive	—	—	—	—	—	—	—	—
Correctly negative	1	2	—	—		1	1	7
False negative	8	6	6	—	—	—	—	70
Number of cases	9	10	16	19	17	16	12	99

Acta Obstet Gynec Scand 53 35–41 1974

PLACENTA PRAEVIA AND ABRUPTIO PLACENTAE

Clinical Experience with Placental Scintigraphy and an Afterloading Technique for Indicating the Cervix

Hans Gustafson Sverker Nordlander Bjorn Westin and Per Erik Åsard

From the Department of Gynaecology (Head, Professor Harald Johansson M.D.), the Radiodiagnostic Department (Head S Nordlander M.D.) and the Department of Medical Physics (Head Per-Erik Åsard, Ph D.) Danderyds Hospital, Danderyd S eden

Abstract Placental scintigraphy with ^{113m}In (Indium) combined with cervical marking with shielded ^{60}C (Cobalt) radioactive source is easy to perform and causes no harm to the patient The radiation dose to mother (gonads, about 15 mrad) and fetus (about 10 mrad) extremely low A total of 111 patients have been examined The method provides accurate results in localizing the placenta Low implantation or placenta praevia were diagnosed in 45 patients Scintigraphic evaluation of disturbances in placental blood flow has given promising results (16 patient). In great number of cases it has been possible to avoid unnecessary and expensive hospitalization by excluding placenta praevia as cause of vaginal bleeding It is reliable method for localizing the placenta before diagnostic amniocentesis

Bleeding during late pregnancy is fairly common symptom which occurs in approximately 3% of all pregnancies In all such cases the obstetrician wants to know the site of the placenta The most important cause of bleeding is placenta praevia. In the remaining cases one may suspect impaired placental blood flow g in abruptio placentae which xcept in advanced cases, is difficult to diagnose ith available clinical methods Different methods such as plain film radiography placentography arteriography ultrasound placentography and ther isoplacentography have been used to localize the placenta (3 7) Pelvic angiography i probably the most accurat method of placental localization The method is how rather e pensiv time consuming p es high adiation dose to the fetus and not free from maternal discomfort and complications The gamma camera today available in many hospital If marking of the internal cervical os were to be added, scintigraphic placental localization using ^{113m}In should be a reliable method with many advantages, amongst others low radiation dose to mother and fetus. The present paper is report of our experience with this technique

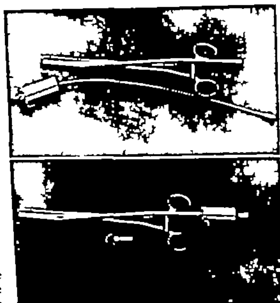


Fig 1 The tenaculum used for cervical marking with the steeltube soldered on it The safety container with its ^{60}Co-source is applicated on the tube in the lower part of the figure When the container is removed, the plug, made of steel and lead seen in the figure is inserted to protect from radiation

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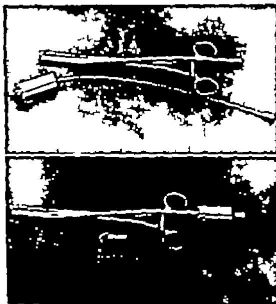


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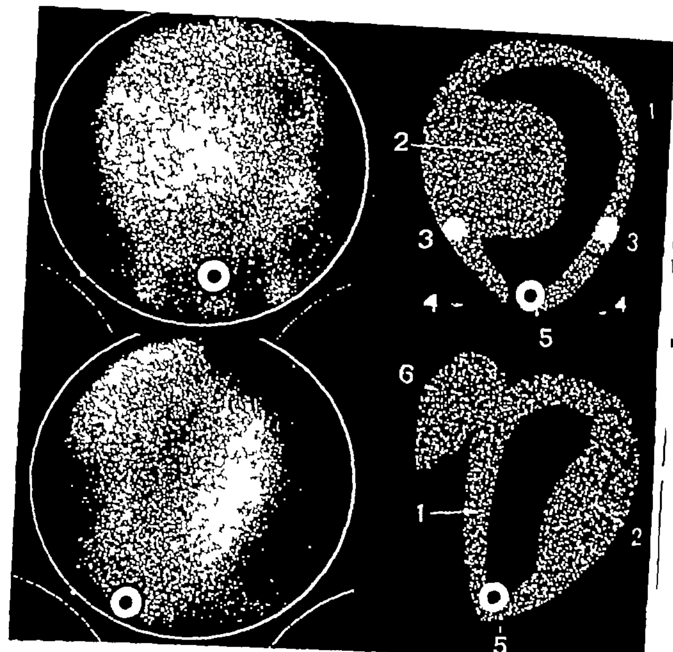


Fig 2 Normal scintigrams in frontal (above) and lateral (below) view showing placenta anteriorly on the right side of the uterus. Schematic drawings to the right.

1 Uterine wall 2 Placenta 3 Uterine vessels. 4 Femoral vessels. 5 Marking of the internal os of the cervix 6, Liver

MATERIAL AND METHODS

Gamma camera

We use a Pho Gamma III (Nuclear Chicago) with diverging collimator and every patient is examined in a frontal and a lateral projection and sometimes, if necessary in both lateral projections. The oscilliscopic view is pictured by a polaroid camera

Radionuclide

Several different radionuclids, such as ^{131}I and ^{99m}Tc have been used for placental localization (4 10) We use ^{113m}In-chloride without stabilizer which easily can be prepared from a generator containing ^{113}Sn (12).

^{113m}In has a short half life (about 100 min) which makes it safe for both patient and fetus (12).

Following intravenous injection ^{113m}In is almost completely bound to transferrin and a negligible amount of activity less than 0.5% will pass the placental barrier to the fetus (1 12, 13) Blocking of the maternal and fetal thyroid is not necessary Due to minimal renal excretion confusing activity from the bladder will not appear

1 mCi of ^{113m}In is given to the patient There is a considerable difference in maternal and fetal radiation dose in favour of scintigraphy when comparing scintigraphy with x-ray methods, especially angiography The absorbed fetal dose using pelvic angiography for placental localization is about 1000

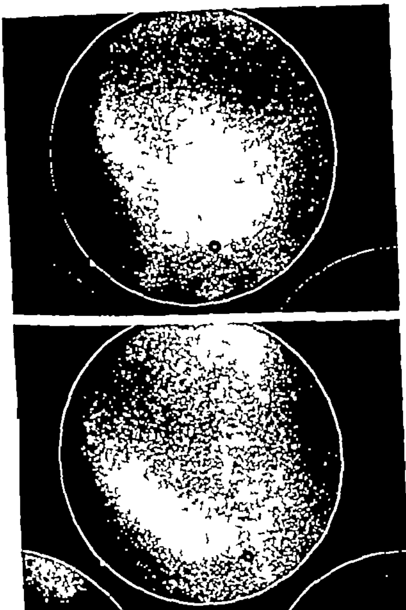


Fig. 3. Frontal (above) and lateral scintigrams from patient with total placenta praevia. In both projections the marked internal cervical os is completely covered by the placenta.

mrad. Corresponding fetal dose for scintigraphy (1 m C ^{113m}In) is only about 10 mrad (12) and for maternal gonads about 15 mrad (12).

Marking of internal cervical os

In many cases it is impossible to distinguish between marginal and total placenta praevia on scintigram unless direct marking of the internal cervical os has been performed. Marking of the umbilicus or the symphysis pubis has been shown to give very incorrect results (9).

We use an afterloading technique with ^{60}C as radioactive source for cervical marking (16). On the cervical shank of special tenaculum stainless steel tube is soldered. The tube is closed at it end by plug made of steel and lead protecting the surrounding tissues from unnecessary radiation (Fig. 1). The tenaculum is fastened to the posterior lip of the cervix as part of routine vaginal examination before scintigraphy. After finishing each view of the placenta the radioactive source is pushed into the tube for few seconds thus marking point close to the internal os.

This method of cervical marking is easy to handle in routine work and gives maximum radiation dose

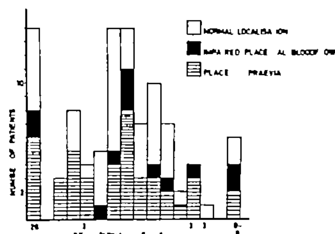


Fig 4 Distribution of patients in bleeding group according to gestational length Separate marking for normal scintigrams cases of placenta praevia and verified impaired placental bloodflow

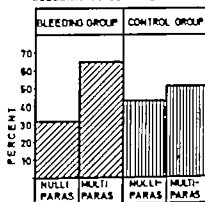


Fig 5 Comparison of parity in bleeding group (n 96) and control group (n=3260) The difference probable ($p<0.05$)

to the fetus of less than 0.5 mrad (16). Figs and 3 illustrate and explain a normal scintigram and give an example of a total placenta praevia

Material

111 pregnant patients have been examined In 99 cases the cause of examination was vaginal bleeding The remaining patients were examined before amniocentesis (3 patients) on account of acute abdominal pains with suspicion of abruptio placentae (3 patients) and because of abnormal presentation (6 patients)

RESULTS

Bleeding group

The distribution according to gestational length is shown in Fig. 4 There is a maximum around 32–33 weeks of pregnancy Fifty per cent of all examined patients had abnormal scintigrams—either placenta praevia or blood flow disturbances usually partial placental separation There was a larger proportion of abnormal scintigrams in the group of patients who got symptoms in early pregnancy compared with those in late pregnancy These patients obviously belong to an obstetrical high-risk group

The birth weight was less than 500 g in 30% of the cases In a control group comprising 3 170 deliveries from the same period only about 3% of all newborn babies weigh less than 2 500 g The difference is highly significant ($p<0.001$)

Table I shows that approximately half of the patients with bleeding in early pregnancy had babies with birth weights less than 500 g. The same proportion for patients in late pregnancy was only 18%. The difference is highly significant ($p<0.001$).

Fig 5 illustrates an increased risk of bleeding in multiparas as compared with nulliparas ($p<0.05$). A reliable determination of the site of the placenta can only be performed in cases when pregnancy is terminated by Caesarean Section (C S) From the clinical point of view an indirect criterion of correct diagnosis is given by studying the site of rupture of the fetal membranes and by normal delivery

Table I *Risk for low birth weight in patients with bleeding during early versus late pregnancy*

Gestational period	Less than 2 500 g (%)	More than 500 g (%)
Before 32 weeks	48	52
After 32 weeks	18	82

Table II *Placental localisation in 97 patients Scintigraphic versus clinical findings*

Placental localisation	Cor rect	Consistent with scint	Partially correct	No of patients
Scintigraphy				
Normal	10	42		5
Low implantation		18		18
Marginal implantation	1	8	2	2
Total praevia	4		1	5

One case estimated a a total praevia at vaginal examination before C.S The other patient s scintigram wa performed before using the [I]Co-indication-technique and proved to be a total praevia
[b] C.S was performed-placenta estimated as marginal

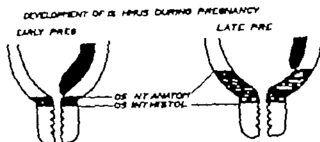


Fig. 6. Variation in placental localization during pregnancy—a function of the development of the isthmus part of the pregnant uterus

about placental complication in cases with a clinical suspicion of placenta praevia. Successful abdominal amniocentesis following placental scintigraphy gives as well an indirect evaluation of the method.

On reviewing the scintigrams we have distinguished between the following placental sites: normal, low implantation, marginal or total placenta praevia. Low implantation means insertion in the lower segment of the uterus. The development of the lower uterine segment is here of great importance. The isthmic part of a nonpregnant uterus has a length of about 1 cm. There is a continuous growth of the lower uterine segment during pregnancy and at term it is about 10 cm in length.

Fig. 6 illustrates the possible variation in placental localisation in the same patient depending on actual length of pregnancy. Consequently a placenta estimated to be total or marginal placenta praevia by early examination may at term be marginal or a low implantation. This means a shift toward a lower obstetrical risk group and may influence the mode of delivery.

A comparison between scintigraphic and clinical findings concerning placental localisation is made in Table II. The group "correct" is almost equivalent to patients delivered by C.S. Normal deliveries are mainly in the group "consistent with scint." Table III summarises the results in 96 patients where the cervical indicator technique was used. There is excellent agreement between scintigraphic and clinical findings.

In the groups with low and normal implantation of the placenta the frequency of C.S. was relatively low, about 20% (Table IV). The "normal" group includes a number of cases with disturbances in placental blood flow responsible for the rise in the frequency of C.S. from our normal 5%. The high frequency—50%—of C.S. in cases of marginal placenta praevia is notable. The reason for C.S. has in almost every case been further haemorrhage and fetal distress.

Scintigraphic evaluation of placental blood flow

Table III. *Placental localisation in 96 patients using 'C-indication of the internal cervical os with afterloading technique*

Placental localization	No of patients	Clinical findings consistent with scintigraphy (%)
Normal		100
Low implantation	18	100
Marginal or total placenta praevia	76	97*

Table IV. *Way of delivery. Frequency of normal delivery resp. C.S. in different groups of placental localization*

Placental localization	Normal delivery (%)	Caesarean section (%)
Scintigraphy		
Normal	80	20
Low implantation	83	17
Marginal	50	50
Total praevia		100

Table V. *Scintigraphic evaluation of disturbances in blood flow through the placenta. Material divided according to scintigraphic finding of small or large defect in isotope uptake*

Scintigraphic findings	Correct	False positive	False negative
Small defects	3	4	3
Large defects	18	3	

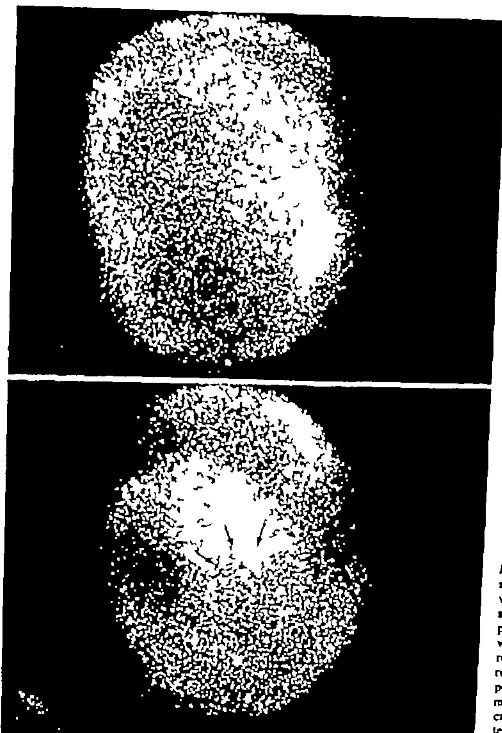


Fig 7 Frontal and lateral scintigrams from a patient with vaginal bleeding as only symptom In the lateral projection there is an obvious perfusion defect (arrows) clinically corresponding to a partial placental separation The marking on the picture indicates the position of the internal os.

disturbances usually partial abruptio placentae has along with increasing experience given promising results. Table V is an attempt to divide the material into small and more obvious disturbances from normal isotope uptake Most of these patients with flow disturbances had bleeding as their only symptom The scintigraphic finding has often been a surprise and at the same time of great help in the care of these patients

A scintigram with an obvious isotope defect in the lateral projection is shown in Fig. 7 The clinical finding was that of a partial placental separation

Other indications for scintigraphy

In a few cases we have used placental scintigraphy before therapeutic and diagnostic amniocentesis Transabnormal amniocentesis could then be performed without complications Acute abdominal pains in late pregnancy may often cause differ

ential diagnostic problems. In three actual cases scintigraphic placental localisation helped in excluding abruptio placentae as cause of the pains (placenta to the left, pains to the right etc.) Placenta praevia is a possible cause of abnormal presentation. In six cases of transverse lie the placenta was found in normal position on the scintigram. Normal delivery was the result in five cases. C.S. was performed in one patient who went into labour with a persistent transverse lie.

DISCUSSION

^{113m}In has proved to be a very suitable isotope for clinical use in placental scintigraphy (2, 6, 8, 10, 14). It is readily available and easy to handle. It gives no confusing activity from the bladder. High doses of iron given a relatively short time before the examination may cause transferrin saturation. Under such circumstances ^{113m}In binds to alpha-globulins with partial urinary excretion (5). This disadvantage has not been observed. With the described technique for marking the internal os of the uterine cervix (16), it has been possible to distinguish between a total and a marginal placenta praevia. There have been no complications using this method. The cervical probe never caused bleeding from major placenta praevia.

The data of Walker (11) concerning bleeding during pregnancy and premature birth are consistent with our findings. In our series bleeding before the 32nd week of pregnancy means a 37 risk of birth weight less than 2 500 g. This holds true independently of placental localization.

It appears that we have been able to discover placental separation and larger placental infarcts with only minimal symptoms. There is however a need for higher resolution in our scintigrams in order to improve this part of scintigraphic placental diagnosis. This may be done by improvement of technical equipment and examination technique.

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REFERENCES

1. Adatepe, M. H., Prakoske, P., van Amberg, A., Wharton, T., Evens, R. G. & Potchen, E. J.: Red cell and plasma protein labeling with ^{113m}In. Int J Appl Radiat Isot 22: 498, 1971.
2. Burdine, J. A., Jr & Werch: ^{113m}In gelatin complex for placental localization. Am J Obstet Gynecol 104: 748, 1969.
3. Drukker, B. H., Reynolds, W. A., Ortiz, E. & Josey, J. C.: Placental localization. A comparative evaluation of isotopic placentography and sonography. Am J Obstet Gynecol 110: 9, 1971.
4. Fisk, M. & Forsman, L.: Placentalokalisation med radioaktiva isotoper. Jordemodern 2: 41, 1972.
5. Hosain, F., McIntyre, P., Poulose, P. A., Stern, H. S. & Wagner, H. N. Jr: Binding of trace amounts of ^{113m}In to plasma transferrin. Clin Chim Acta 24: 69, 1969.
6. Hoddleston, J. E., Mishkin, F. S., Carter, J. E., Dobois, P. D. & Reese, I. C.: Placental Localization by Scanning with ^{113m}In. Radiology 92: 587, 1969.
7. Laakso, K., Kivikoski, A. & Rauramo, L.: Placental localization by the gamma camera and Doppler flowmeter. Acta Obstet Gynecol Scand 50: 171, 1971.
8. Mishkin, F. S., Carter, J. F. & Reese, I. C.: Placental imaging with ^{113m}In. Am J Roentgenol 109: 776, 1970.
9. Rosenthal, L.: Intravaginal markers for accurate radionuclide localization of the placenta. Am J Roentgenol 106: 787, 1969.
10. Urbanek, Graf M.: Placental scanning using ^{99m}Tc-albumin and ionic ^{113m}In. J Nucl Med 12: 825, 1971.
11. Walker, J.: Clinical obstetric features of prematurity, gestational age, size and maturity (ed. Dawkins & McGregor). London Spastics Soc. (1965).
12. van der Merve, E. J., Lotter, M. G., van Heerden, D. D. R., Slabber, C. F. & Bester, J.: Absorbed dose calculations for ^{113m}In placental scanning. J Nucl Med 11: 31, 1970.
13. Wochner, R. D., Adatepe, M., van Amburg, A. & Potchen, E. J.: A new method for estimation of plasma volume with the use of the distribution space of ^{113m}In-transferrin. J Lab Clin Med 75: 711, 1970.
14. Wright, F. W.: Placental localisation by isotope scanning with ^{113m}In. Results in 200 patients. Br Med J 1: 636, 1970.
15. Würtner, Kl., Leven, H. & Grisslin, D.: Über die Strahlenbelastung bei der Verwendung von ^{113m}In zur Plazentadarstellung. Fortschr Geb Roentgenstr Nuklearmed 112: 478, 1970.
16. Åsard, P.-E., Gustafson, H., Nordlander, S. & Westin, B.: A technique for the localization of the internal cervical os in placental scintigraphy. Acta Radiol 12: 452, 1972.

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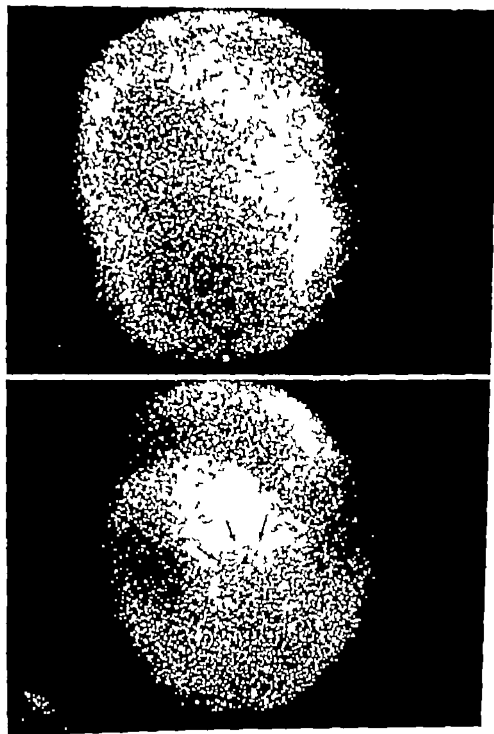


Fig 7 Frontal and lateral scintigrams from a patient with vaginal bleeding as onl[y] symptom In the lateral projection there is an obvious perfusion defect (arrows) clinically corresponding to a partial placental separation The marking on the picture indicates the position of the internal os

disturbances usually partial abruptio placentae has along with increasing experience given promising results Table V is an attempt to divide the material into small and more obvious disturbances from normal isotope uptake Most of these patients with flow disturbances had bleeding as their only symptom The scintigraphic finding has often been a surprise and at the same time of great help in the care of these patients.

A scintigram with an obvious isotope defect in the lateral projection is shown in Fig 7 Th[e] clinical finding was that of a partial placent[al] separation

Other indications for scintigraphy

In a few cases we have used placental scintigraph[y] before therapeutic and diagnostic amniocente[sis] Transabdominal amniocentesis could then b[e] performed without complications Acute abdomin[al] pains in late pregnancy may often cause diffe[...]

Acta Obstet Gynec Scand 53 43–246 1974

CHANGES IN HEMOGLOBIN CONCENTRATION AND HEMATOCRIT DURING THE MENSTRUAL CYCLE

I A Cross sectional Study

Odd D Vellar

From the I stitute of Hygiene (Head Prof Haakon Nat n) University of Oslo Oslo Norway

Abstract In series of 477 omen of child-bearing age altogether 1270 blood samples w re tested for hemoglobin hematocrit and mean corpuscular hemoglobin concentration (Hb Hct and MCHC) and the results plotted against normal menstrual cycle There as pronounced tendency towards an increase of Hb together th Hct from the early menstrual phase until the post ulatory period th subsequent decrease towards t end of the cycle No cyclical pattern of MCHC w s served These ariations in hematological parameters t in good agreemen with present knowledge of the rmonal hanges during the menstrual cycle

he rhythmic or cyclical nature of most if not all iological processes has long been accepted he best-known rhythm is the diurnal or 24-hour ctivity cycle The rhythmic nature of the menstrual ycle in women is also very well documented

Variations in body-weight during the menstrual ycle have been studied by a number of workers ith conflicting results (16) Recent studies however have revealed an increase in body-weight and/ or other ymptoms of oedema in a relati ely high proportion of omen during the premenstrual and early menstrual phase (4 9 13 16, 17), although the recorded weight increases usually have been ery slight (4 9 16).

It might be expected that the fluid accumulation during the premenstrual and early menstrual phase would cause hemodilution with a subsequent fall in the level of hemoglobin and certain other hematological parameters Such hemodilution has been reported in individual cases by Katharina Dalton (2, 3) a well a in a small material of French omen (14).

The object of the present cross-sectional study was to examine in a large series of women if there was any pattern of fluctuations in hemoglobin concentration (Hb) and hematocrit (Hct) during the menstrual cycle, thus providing additional presumptive evidence of cyclical fluid accumulation.

MATERIAL AND METHODS

The material consisted of total of 477 women of child-bearing age: 427 were female employees (mean age 32 years) participating in the Norwegian voluntary industrial health service program, and 50 were physical education students (mean age 22 years) The female employees lived in Stavanger and Bergen and were examined in connection with long-term community-based experiment with ironfortified bread (7). The students who lived in Oslo were xamined in connection with study of the relationship between physical performanc and hematological parameters (15)

In the female employees blood was obtained from finger-tip by pricking with lancets. In each of these subjects, one to five blood samples were taken during pe riod of about two years. All the samples were taken and the readings performed by the same well-trained nurse

I the students the blood samples wer collected in heparinized centrifuge tubes after venepuncture of the antecubital vein The same physician (the author) performed the venepuncture in all cases Only one blood sample from each student is included in this study

The Hb determinations were performed by the cyanmethemoglobin method with photoelectric reading in a Lumson Junior photoelectric colorimeter The colorimeter was calibrated against standardized cyanmethemoglobin solutions. The Hct was measured in heparinized capillary tubes after centrifugation in hematocrit centrifuge (AB L. Ljungberg & Co Stockholm) The analytical methods were in principle consistent with the standard procedure used in our Institute during the past several years (6)

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CHANGES IN HEMOGLOBIN CONCENTRATION AND HEMATOCRIT DURING THE MENSTRUAL CYCLE

I A Cross-sectional Study

Odd D Vellar

From the Institute of Hygiene (Head Prof Haakon Natvig) University of Oslo Oslo Norway

Abstract In a series of 477 women of child-bearing age together 1 270 blood samples were tested for hemoglobin hematocrit and mean corpuscular hemoglobin concentration (Hb, Hct and MCHC) and the results plotted against normal menstrual cycle There was pronounced tendency towards an increase of Hb together with Hct from the early menstrual phase until the post ovulatory period, with a subsequent decrease towards the end of the cycle No cyclical pattern of MCHC was observed These variations in hematological parameters are in good agreement with present knowledge of the hormonal changes during the menstrual cycle

The rhythmic or cyclical nature of most, if not all biological processes has long been accepted The best-known rhythm is the diurnal or 24-hour activity cycle The rhythmic nature of the menstrual cycle in women is also very well documented

Variations in body-weight during the menstrual cycle have been studied by number of workers, with conflicting results (16). Recent studies however have revealed an increase in body weight and/or other symptoms of oedema in a relatively high proportion of women during the premenstrual and early menstrual phase (4 9 13 16, 17) although the recorded weight increases usually have been very slight (4 9 16).

It might be expected that the fluid accumulation during the premenstrual and early menstrual phase would cause hemodilution with a subsequent fall in the level of hemoglobin and certain other hematological parameters. Such a hemodilution has been reported in individual cases by Katharina Dalton (2, 3) as well as in a small material of men (14).

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The object of the present cross-sectional study was to examine in a large series of women if there was any pattern of fluctuations in hemoglobin concentration (Hb) and hematocrit (Hct) during the menstrual cycle thus providing additional presumptive evidence of cyclical fluid accumulation.

MATERIAL AND METHODS

The material consisted of total of 477 women of child-bearing age 427 were female employees (mean age 32 years) participating in the Norwegian voluntary industrial health service program and 50 were physical education students (mean age 22 years). The female employees lived in Stavanger and Bergen and were examined in connection with long-term community-based experiment with nonfortified bread (7). The students, who lived in Oslo were examined in connection with study of the relationship between physical performance and hematological parameters (15)

In the female employees blood was obtained from a finger-tip by pricking with lancets In each of these subjects, one to five blood samples were taken during period of about two years All the samples were taken and the readings performed by the same well-trained nurse

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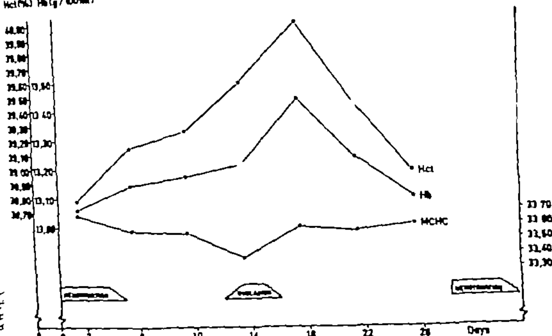


Fig. 1. The changes in hemoglobin concentration (Hb), hematocrit (Hct) and mean corpuscular hemoglobin concentration (MCHC) during the menstrual cycle. The values are based on means of four days. The assumed average time of menstruation and ovulation is indicated.

of the cycle. The difference between the highest 4 day mean (39.98 %) and the lowest (38.78 %) is 1.2 % or 3.1 % of the mean value for the entire 28 day period.

The MCHC value, which are based on the mean values for Hb and Hct in each 4 day period, are shown in Fig. 1. Apparently, there are only minor fluctuations in the MCHC values. Furthermore, if the mean MCHC values are based on periods of one week instead of 4 days, the fluctuations due to the relatively high value at the start of the cycle and the relatively low value at the time of ovulation are eliminated. Thus the constant level of the MCHC value is quite different from the cyclical pattern of Hb and Hct whose trends are identical regardless of whether 4 day means or weekly means are used for comparison.

DISCUSSION

The observed changes in Hb and Hct are in good accordance with present knowledge concerning hormonal fluctuations during the menstrual cycle. The post-ovulatory hemoconcentration (maximum at the 18th day) might be explained by the increase in progesterone during the luteal phase as progesterone is a potent anti-aldosterone (5). The subsequent relative hemodilution in the premenstrual phase might be due to the compensatory increase in aldosterone later in the luteal phase (8, 11, 12). Furthermore, as it has been shown that plasma angiotension II concentration is increased during this phase of the cycle (11), it is also possible that vasopressin is increased, as angiotensin II stimulates vasopressin secretion (1, 10).

The actual loss of erythrocytes during menstrual bleeding probably also contributed slightly to the hemodilution as the lowest values of Hb and Hct were recorded during the bleeding phase. However, hormonal factors seemed to be more responsible as values which were nearly as low were observed just before menstruation.

Hematological variations during the menstrual cycle, observed in this cross-sectional study are now being examined longitudinally in more detail in a series of healthy young adult female students. Serum iron, total iron binding capacity, serum B_{12} and folic acid will also be included.

Table I *Hemoglobin concentration (Hb) during the course of the menstrual cycle*

Days after start of menstruation	No of Hb observations	Hb (g/100 ml) Mean per day	Hb (g/100 ml) Mean in each period of 4 days
0	17	13 05	
1	51	12 99	13 06
2	50	12 90	
3	48	13 30	
4	55	12.94	
5	48	13.32	13 14
6	56	12 93	
7	62	13.37	
8	44	13 11	
9	44	13 22	13 17
10	47	13 21	
11	45	13 13	
12	57	13.25	
13	46	13.28	13.21
14	50	13 03	
15	49	13.26	
16	53	13.28	
17	53	13.51	13 43
18	45	13.57	
19	52	13.37	
20	38	13 23	
21	36	13 11	13.23
22	43	13 24	
23	49	13.32	
24	43	13 05	
25	32	13 11	13 09
26	33	12 98	
27	24	13.29	
Total cycle (28 day period)	1 270	13 20	

When the blood specimens were taken the first day of the last menstrual bleeding was recorded in each individual By subtracting the date of the first day of the last menstrual bleeding from the date of the blood examination the number of days since the start of the last menstrual bleeding was calculated In the further analysis only observations related to days 0–27 in the menstrual cycle (a normal" period of 28 days) were included The total number of Hb observations according to these criteria, were 1 270 (Table I). The total number of Hct determinations however were 1 269 (Table II) as the determination failed in one blood sample.

RESULTS

Hb concentration

There are appreciable fluctuations in the daily mean Hb values during the course of the menstrual cycle (Table I) However there is a tendency towards increase in the values until about the 18th day with a subsequent decrease towards the end of the cycle Consequently when means per 4 day periods are calculated this trend is clearly demonstrated. The difference between the highest 4 day mean (13 43 g/100 ml) and the lowest (13 06 g/100 ml) is 0 37 g/100 ml or 2 8 % of the mean value of the entire 28 day period

The Hct values (Table II) agreed rather well with the Hb values When the means per 4 day periods are calculated there is an obvious tendency towards an increase during the first five 4 day periods with a subsequent decrease towards the end

Table II *Hematocrit (Hct) during the course of the menstrual cycle*

Days after start of menstruation	No of Hct observations	Hct (%) Mean per day	Hct (%) Mean in each period of 4 days
0	17	39 06	
1	51	38.77	38.78
2	50	38.26	
3	48	39.23	
4	55	38 62	
5	48	39.67	39 14
6	56	38 63	
7	62	39 65	
8	44	39 02	
9	44	39 46	39.25
10	46	39 11	
11	45	39 40	
12	57	39 95	
13	46	39 94	39.58
14	50	38.88	
15	49	39.53	
16	53	39.87	
17	53	40 06	39 98
18	45	40 09	
19	52	39 90	
20	38	39 16	
21	36	38.81	39 42
22	43	39.51	
23	49	40.00	
24	43	39 12	
25	32	38 97	38.96
26	33	38.36	
27	24	39.50	
Total cycle (28 day period)	1 269	39.33	

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CONSTRUCTION OF A CLOSURE MECHANISM FOR THE EXTERNAL URETHRAL ORIFICE IN WOMEN—A METHOD TO TREAT PATIENTS WITH RECURRENT URETHRITIS FOLLOWING COITUS

Axel Ingelman-Sundberg

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Abstract. An operation is described for women with ide external urethral meatus, suffering from recurrent urethritis following intercourse. It consists of the formation of structure similar to the frenulum of the prepuce in the male. On pulling this band the external urethral orifice will close. This will happen at penetration by the penis

tion. With this in mind, I started to investigate the possibilities of constructing a mechanism similar to the frenulum of the prepuce in the male After several different attempts the following operation was worked out. No similar procedure has been found in the literature available

Urethritis is a common disease in women if properly diagnosed. In our gynaecological department about 4% of the patients suffe from chronic or recurrent urethritis and among the out-patients the figure is higher

In some of these women diverticulae can be demonstrated by urethroscopy or urethrography The patients are as a rule cured after excision of the diverticulae

There is also a group of climacteric and post climacteric women who are easily cured following conventional therapy but who get recurrences when they resume intercourse In some of the cases the husband has a urethritis which must be treated but there are patients, where normal vaginal bacteria seem to cause the urethritis through being forced into the urethra during coitus I few of these the external urethral orifice is very narrow hich seems to counteract the normal rinsing of the distal urethra at micturition. They are usually cured following meatotomy But there are also women, who have a wide external urethral meatus sometimes with caruncle formation. If in these cases, mechanism could be constructed, which closes the female external urethral orifice at the introduction of penis, it might prevent the infec

OPERATIVE TECHNIQUE

1 A transverse incision is made along the n terior vaginal circumference of the external urethral orifice If a caruncle is present it is excised (Fig 1)

2 The anterior vaginal wall is dissected free from the urethra for a length of about 25 mm.

3 From the tissue beneath the vaginal epithelium a trouser-like structure is formed, about 15 mm long and 2 mm thick Each leg should be 3 mm wide at the base and about 10 mm long. A chromic catgut 0000 suture is placed in the tip of each leg, whereupon this is trimmed laterally to width of about 1 mm at the end The proportions must, however be varied according to the local circumstances in each individual case.

4 A tunnel is made under the kin of the introitus with pair of scissors as shown in Fig. 4

5 The legs of the new frenulum are brought through the tunnel each one into the corresponding incision in the lateral circumference of the external urethral meatus and sutured superficially in the posterior half of the incision at its closure.

6. The transverse incision under the external urethral meatus is closed

REFERENCES

1. Bonjour J. P. & Malvin R. L. Stimulation of ADH release by the renin-angiotensin system. Amer J Physiol 218: 1555, 1970.
2. Dalton K. Discussion on the aftermath of hysterectomy and oophorectomy. Proc Roy Soc Med 50: 415, 1957.
3. — The premenstrual syndrome. William Heinemann Medical Books Ltd., London 1964.
4. Golub L. J., Menduke H. & Conly S. S. Jr: Weight changes in college women during the menstrual cycle. Amer J Obstet Gynecol 91: 89, 1965.
5. Landau R. L. & Lugibihl K. The catabolic and natriuretic effects of progesterone in man. Recent Progr Hormone Res 17: 249, 1961.
6. Natvig H. (ed.) Hemoglobin values in Norway. Universitetsforlaget, Oslo 1969.
7. Natvig H. & Vellar O. D. Iron fortified bread. A long-term controlled community-based experiment with ferrous sulphate-enriched flour. Acta Med Scand. In press.
8. Reich M. The variation in urinary aldosterone levels of normal females during the menstrual cycle. Aust Ann Med 11: 41, 196..
9. Robinson M. F. & Watson, P. E. Day-to-day variations in body weight of young women. Br J Nutr 19: 225, 1965.
10. Severs W. B. Central effect of angiotensin: release of pituitary pressure material. J Pharmacol Exp Ther 174: 27, 1970.
11. Sundsfjord J. A. & Aakvaag A. Plasma angiotensin II and aldosterone excretion during the menstrual cycle. Acta Endocrinol (Kbh) 64: 452, 1970.
12. — Variations in plasma aldosterone and plasma renin activity throughout the menstrual cycle, with special reference to the pre-ovulatory period. Acta Endocr (Kbh) 73: 499, 1973.
13. Timonen S. & Procopé B. J. Premenstrual syndrome and physical exercise. Acta Obstet Gynecol Scand 50: 331, 1971.
14. Vartiainen E. & Zilliacus, H. Red and white blood counts during the menstrual cycle. Ann Chir Gynaec Fenn 57: 287, 1968.
15. Vellar O. D. & Hermansen, L. Physical performance and hematological parameters. With special reference to hemoglobin and maximal oxygen uptake. Acta Med Scand. Suppl. 5.., 1971.
16. Watson P. E. & Robinson M. F. Variations in body weight of women during the menstrual cycle. Br J Nutr 19: 237, 1965.
17. Widholm O. & Kantero R. L. A statistical analysis of the menstrual patterns of 8 000 Finnish girls and their mothers. III. Menstrual patterns of adolescent girls according to the chronological and gynecological ages. Acta Obstet Gynecol Scand, Suppl. 14, 1971.

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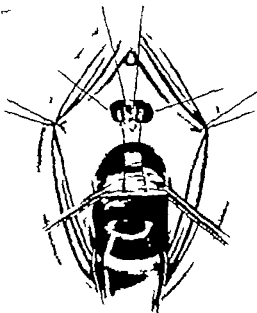


Fig 5 The legs of the new frenulum are brought through the tunnel, each one into the corresponding incision in the lateral circumference of the external urethral meatus. They are stitched in the posterior part of the incision.

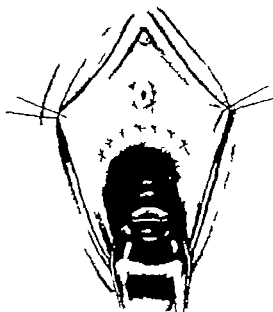


Fig 6 The incisions are closed.

RESULTS AND CONCLUSIONS

This operation was carried out 1966 in two women of 32 and 33 years of age suffering from recurrent urethritis following intercourse. At follow-up three years later both had been completely free from symptoms since the operation. Because of this promising result I thought it justifiable to try the procedure in additional cases. So far five new patients have been operated on, two of which also had caruncles, which were excised at the same time. In three the results were good and two were failures because the frenulum did not function properly. I therefore consider that a trial of this small, but rather delicate operation in suitable cases is justified. The selection of patients must however be carefully made and postmenopausal women must be given estrogens as well.

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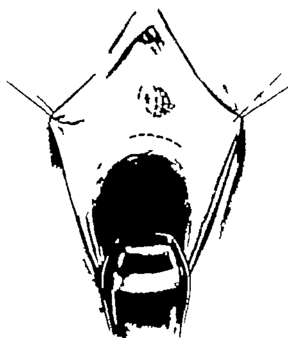


Fig 1 A transverse incision is made according to the figure Two small incisions are made in the lateral circumference of the external urethral orifice If a caruncle is present it is excised

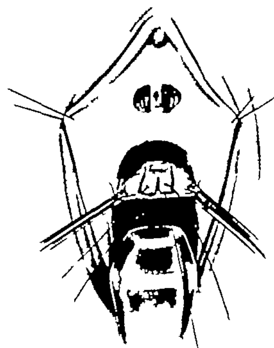


Fig 3 A chronic catgut 0000 suture is placed in the tip of each leg, cut according to Fig. 2 whereupon the leg is trimmed laterally to a width of about 1 mm at each end The proportions must however be varied, according to local circumstances in each individual case

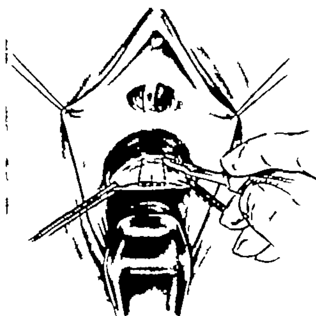


Fig 2 The anterior vaginal wall has been dissected free from the urethra at a length of about 25 mm From the connective tissue beneath the vaginal epithelium a trouser-like structure is formed about 15 mm long and 2 mm thick. Each leg should be 3 mm wide at the base and about 10 mm long.

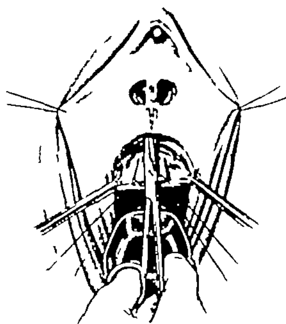


Fig 4 A tunnel is made under the skin of the introitus with a pair of scissors

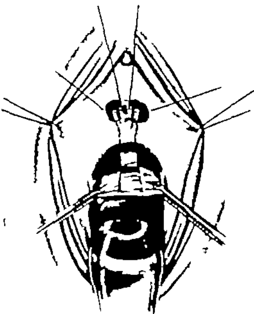


Fig. 5. The legs of the new frenulum are brought through the tunnel, each one into the corresponding incision in the lateral circumference of the external urethral meatus. They are stitched in the posterior part of the incisions.

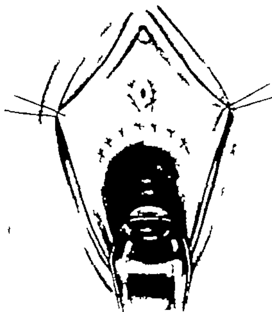


Fig. 6. The incisions are closed.

RESULTS AND CONCLUSIONS

This operation was carried out 1966 in two women of 32 and 33 years of age suffering from recurrent urethritis following intercourse. At follow-up three years later both had been completely free from symptoms since the operation. Because of this promising result I thought it justifiable to try the procedure in additional cases. So far five new patients have been operated on, two of which also had caruncles which were excised at the same time. In three the results were good, and two were failures, because the frenulum did not function properly. I therefore consider that a trial of this small, but rather delicate operation in suitable cases is justified. The selection of patients must, however, be carefully made, and postmenopausal women must be given estrogens as well.

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A STUDY OF SEASONAL AND SECULAR TRENDS IN INCIDENCE OF STILLBIRTHS AND SPONTANEOUS ABORTIONS IN SWEDEN

Björn Sandahl

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Abstract An investigation was made of secular and seasonal trends in two aspects of reproductive failure: stillbirth and spontaneous abortion. The study of stillbirths during 1965 to 1971 is based on official statistics for the whole of Sweden, representing a total of 3 371 stillbirths among 445 343 births. The date of last menstrual period (LMP) was estimated for the main material from data obtained from a smaller series of [illegible] stillborn infants in Malmö. Spontaneous abortion was studied in the population of Malmö during 1969 to 1971. The yearly number shows a decrease from [illegible] in 1969 to 330 in 1971. The trend towards performing legal termination of pregnancies at earlier gestation periods means that more terminations are being performed on patients who might subsequently have aborted spontaneously.

After correcting for this the number of spontaneous abortions was constant in 1969 389, in 1970 391 and in 1971 390. The total number of pregnancies in Malmö was 3 345, 3 511 and 3 360 respectively. Incidence of stillbirth and spontaneous abortion was determined according to month of LPM.

A decrease in stillbirth rate was observed after 1967 particularly in the high maternal age groups (over 35 years). A seasonal rhythm was noticed with a high stillbirth rate among pregnancies with maternal LMP around March and a low rate in women with their LMP during the summer. A high stillbirth rate is correlated with low pregnancy rate. Spontaneous abortions show a different pattern—with a high rate of spontaneous abortion among pregnancies in women with their LMP around June or at the end of the year. There is apparently no correlation between the seasonal changes in stillbirths and spontaneous abortions.

Causes of human reproductive failure are numerous and complex. The extreme importance of the genetic background is well known. The deleterious action of certain specific diseases, chemicals, and other environmental factors, has also been demonstrated. Much work has been done to identify other and less specific environmental factors that can influence the outcome of a pregnancy. In this connexion interest has been paid to seasonal and secular trends in human reproduction. Analysis of such data may offer hints to causes of reproductive failure. Slatis & de Cloux (13) were first to make a detailed study of seasonal variations in stillbirth frequencies in the USA. A highly seasonal pattern of occurrence was observed with a high peak during April to June and a low peak during the last months of the year. Different populations in the USA differed somewhat in this respect—the most pronounced effects of season were seen in Negro and Southern white populations. The authors stated that a spring-summer low and autumn-winter high stillbirth rate is found in England and Wales resembling that described by Finnish workers (9). Further studies on this subject have been published among others by Barron (1) from England, Czeizel & Elek (2) from Hungary, Janerich & Garfinkel (4) from USA and McDonald (7) from Canada.

The present study aims at an analysis of some features of human reproduction in the whole of Sweden or part of it: birth rate, stillbirth rate and rate of spontaneous abortion. Mainly seasonal variations are studied, but some comparisons between different years are also made. The study was based on data collected during the period of 1963–1972 and described in greater detail below.

MATERIAL AND METHODS

Three sources of data were used for this study:

1) Official statistics for Sweden (8). From this source, we obtained the total number of births for each month

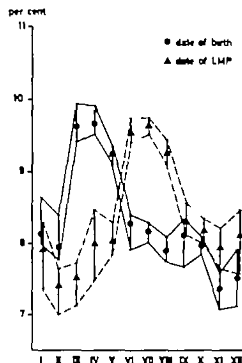


Fig. 1. Distribution (as percentage) of live-born infants in the whole of Sweden 1965–1971 according to month of birth (whole lines) or month of calculated LMP (interrupted lines). For each month maximum minimum+mean percentages are given. Roman numbers on the abscissa indicate the calendar month.

during 1965 to 1971 and the number of stillborn infants and infants dying during the first day of life during 1968 to 1971.

2) A prospective study performed in the city of Malmö during 1963–1964 and comprising appr. 6 400 pregnancies. In all the stated date of LPM (last menstrual period) and the outcome of the pregnancy were known. This data was used for estimating the duration of pregnancies with different outcomes. Details of this study were given by Kullander & Källén (5, 6) and by Ekelund et al. (3).

3) From records of patients at Malmö Allmänna Sjukhus, the only hospital in an area with about 300 000 inhabitants. All women who sought medical assistance in Malmö because of spontaneous abortion, induced abortion or stillbirth during 1969–1972 are included in the investigation. The following sources were searched for information: records on in-patients and out-patients and lists of operations at the Department of Gynaecology; records of biopsies at the Department of Pathology—which also serves private doctors in the city—and records of applications for legal abortions at the Abortion Board of the Department of Psychiatry.

Women who had conceived during 1968 to 1972 were excluded. From the records of the remaining patients information was collected on maternal age, time of LMP and time of abortion or birth. The dividing line between spontaneous abortion and child birth is taken as 29 weeks after the LMP.

Table I. *Duration of pregnancy for 5 606 live-born infants*

Malmö material

Duration of pregnancy weeks after LMP	No. of pregnancies	Percentage, with error
<33	48	0.82±0.12
34–37	347	6.19±0.32
38–42	4 997	89.17±0.42
>43	214	3.82±0.26

RESULTS

1. Live-born infants

The monthly distribution of births is known to be uneven and the pattern differs between different countries. Fig. 1 gives the percentage of each year's live-born infants in Sweden for each calendar month for 1965–1971. The pattern agrees well with the data published by Theander (15) for 1951–1960 and 1961–1965. A χ^2-analysis of heterogeneity between the years 1965–1971 shows a difference in the birth distribution pattern, χ^2 297.51 at 66 d.f. $0.01>P>0.001$. The actual percentage differences between the years are small as Fig. 1 shows. The Swedish birth distribution pattern differs markedly from that found in New York for 1959–1967 (4). The American birth rate peak is in March–September, the Swedish peak in March–April. An autumn peak is just detectable in the Swedish material and similarly a spring peak can be seen in the American material, especially at high birth order. Even at birth order 5+ the autumn peak is higher than the spring peak in the American material.

In order to make the month-of-birth distribution comparable with those found in various types of pathological pregnancies, the distribution was referred to the month of LMP. These dates were not known for the main material, but were known for 5 606 births of liveborn infants from the Malmö material (Table I). With these estimates the monthly distribution of maternal LMP for live-born infants could be calculated (Fig. 1). Maternal age distribution was analysed for the whole of Sweden for the years 1965–1971. Fig. 2*a* shows the percentage in each age class for each year. The graph demonstrates that a steady decrease had occurred in the proportion of very young mothers (under 20 years) and relatively old mothers (over 35 years). Such changes can be due to various causes, fo

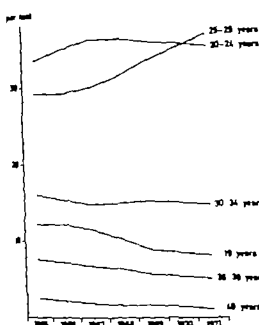


Fig 2 (a) Contribution (as percentage) of different maternal age classes to all births, by years, 1965–1971 whole of Sweden.

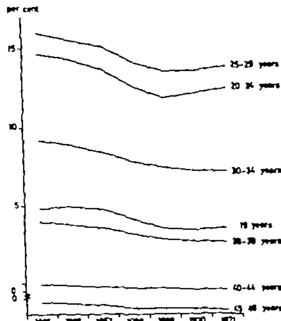


Fig 2 (b). Percentage of women of different age classes who delivered child by years 1965–1971 whole of Sweden

cluding variations in age distribution of all women Fig 2 *b* shows the percentage of all women in each age group who were delivered each year For all seven age groups a downward trend is evident For age groups 20–24 years and 25–29 years this represents a reduction in the probability of childbirth from approximately 0 15 to 0.13 i.e. a reduction of 15% The under 20 years age group shows no change in probabilities for childbirth between 1965–1967 but a marked reduction during 1967–1969 from approximately 0 05 to approximately 0 035 i e of 30% and during 1969–1971 again a steady state Age groups 35–39 40–44 and 45–49 years show a steady decrease in rate amounting to a total of approximately 30–40%

Marriage should increase the probability of childbirth, and marked social changes in this respect should influence birth rates A comparison of the proportions of married women in the different years (Table II) shows a marked decrease in the under 20 years age group during 1967–1971 and only small decreases for other age groups maximally amounting to 5–10% The most likely explanation is that the reduced number of births results in a reduced number of "forced" marriages.

During this period general reduction in childbirth probability thus occurs most pronounced in the very young age group and in the highest age groups (under 20 and over 35 years), reasonably due to better planned pregnancies or increased resort to induced abortions. In the present connexion, the most important feature is the changing age distribution which could be expected to influence stillbirth rates and perhaps also spontaneous abortion rates

2 *Stillbirth*

Stillbirth rate according to month of birth was known for the whole of Sweden for 1968–1971 There were 3 371 stillbirths among 445 343 births. Fig. 3 shows the stillbirth rate among all births according to the month of LMP The date of the LMP was

Table II *Age class distribution*

Percentage of married women 1965–1971

Age class	Jan. 1 1966	Jan. 1 1967	Jan. 1 1968	Dec. 31 1969	Dec. 31 1970	Dec. 31 1971
15–19	4.40	4 42	4 00	[illegible].54	[illegible].33	1 91
20–24	43 48	44.52	44 47	41.20	39 73	36.38
25–29	77.85	77 72	76.98	74.65	73 91	72.27
30–34	85 49	85.27	84 96	83.96	83.57	82.68
35–39	86.19	86.17	85 97	85 43	85.21	84.69
40–44	84.49	84 58	84 77	84 46	84 35	84 09
45–49	82.35	82 41	82.33	82.14	82 06	81 93

Table III *Duration of pregnancy for 181 stillborn infants*
Malmö material

Duration of pregnancy month after LMP	No. of pregnancies	Percentage with error
VIII	43	23.8±6.5
IX	44	24.3±6.5
X	69	38.1±5.8
XI	25	13.8±6.9

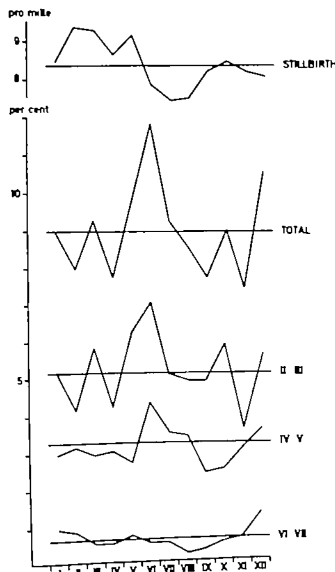


Fig. 3. Top graph: Stillbirth rate per thousand total births, according to calendar month (Roman numbers on abscissa). Whole of Sweden 1965–1971. Lower graphs: Spontaneous abortion rate as percentage of all pregnancies according to calender month. Malmö material 1968–1971. Total=all abortions irrespective of gestational age. Roman figures to the right of the graphs show subdivision of material into three subgroups according to gestational age at abortion.

Table IV χ^2*-analysis of the interaction between two sources of heterogeneity in incidence of stillbirth: maternal age and year of LMP*

Source of heterogeneity	d.f.	χ^2
Total	41	716.0
Between age groups	5	512.9
Between years	6	153.6
Interaction between age groups and years	30	49.5

not known for the main material, but only for 181 stillborn infants from Malmö. The duration of pregnancy found in the latter sample (Table III) made it possible to estimate the distribution of stillbirths according to month of LMP in the main material.

When Fig. 3 is compared with the curve showing the monthly distribution of all liveborn infants (Fig. 1), high stillbirth rates appear to correspond to low incidences of live-born infants. In order to test this statistically, a correlation coefficient was calculated for the percentage of births and the number of stillbirths per 1 000 births each month. When the month of the LMP is used as a basis for the comparison $r=-0.81$, $t=-4.1$, $0.01>P>0.001$. The negative correlation is thus statistically significant. Stillbirth rate is well known to be affected by birth rank and maternal age—these two variables are, of course, closely correlated. Stillbirth rates are plotted in Fig. 4 for three maternal age groups and different years (1965–1971). A χ^2-analysis of the actual numbers of stillborn and live born infants is given in Table IV. It shows that a highly significant interaction exists between

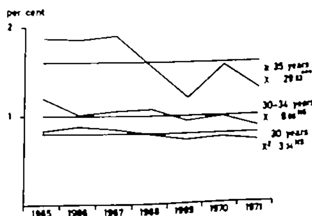


Fig. 4. Stillbirth rates by years for three maternal age classes (<30 years, 30–34 years, ≥35 years).

the two sources of heterogeneity: maternal age and year of birth. Both sources are thus significant and the influence of maternal age varies in different years. Fig. 4 shows than χ^2 for heterogeneity between years is significant only for the age group 35 years and over—but in the other two graphs a trend is seen of continuous decrease in stillbirth rate, even though it is small.

3. Spontaneous abortion

Spontaneous abortions occurring in Malmö during 1969–1971 show a decrease in the annual number from 369 in 1969 to 330 in 1971. Simultaneously the number of induced abortions in Malmö increased from 388 in 1969 to 878 in 1971. These figures refer to the year of the LMP of the pregnancy in question. During the same years a shift has occurred in the policy of legal abortions, leading to earlier operations. This will result in an apparent reduction in the total number of spontaneous abortions, as operation will sometimes be performed on a patient who would otherwise miscarry later on. As the gestational age of 1 970 legal abortions was known in the population, it is possible to correct for this change giving corrected figures of 389 in 1969, 391 in 1970 and 390 in 1971—the total number of pregnancies with LMP in these years in Malmö was 3 345, 3 511 and 3 360 respectively. The mean actual frequency of spontaneous abortions in Malmö during the three years was approximately 9% which is markedly less than the 15% registered by Pettersson (19) in Uppsala. A better identification of cases in the latter study can be one explanation of the discrepancy. However Uppsala University Hospital serves as regional hospital and their figures may therefore be biased by higher proportions of high-risk groups, e.g. *Rh* incompatibility and severe diabetes. The frequency of spontaneous abortions per 100 pregnancies was calculated for each calendar month. A marked peak is found around June and a lesser one around December (Fig. 3). The seasonal variation of spontaneous abortions is statistically significant ($\chi^2=26.8$ at 11 d.f. $0.01>P>0.001$). One explanation for this seasonal variation could be an increase in criminal abortions which were recorded as spontaneous abortion. If this explanation is true, corresponding increase in the rate of legal abortions would be expected. A correlation coefficient was therefore determined between the number of legal abortions and the number of spontaneous abortions for each calendar month. The correlation coefficient was $r=0.18$, $t=0.58$ at 10 d.f. $P>0.05$.

The data has been analysed according to gestational age, as is shown in Fig. 3. A pattern very similar to that found for the total material is seen for abortions occurring at gestational months 2 or 3 and 4 or 5. There is no summer peak observable among abortions taking place at gestational age 6 to 7 months, but a slight increase is seen in December. No significant correlation could be found between spontaneous abortion rate and percentage conceptions for each month—in both cases referred to the month of LMP. The correlation coefficient is -0.48, $t=1.73$ at 10 d.f. $P>0.05$. Spontaneous abortion rate and stillbirth rate do not correlate when compared with respect to LMP ($r=-0.22$, $t=-0.7$, $P>0.05$).

DISCUSSION

The stillbirth rate decreased in Sweden during 1965–1971. Analysis by maternal age showed that the decrease is mainly—but perhaps not exclusively—located to the age group over 35 years. The reason for the change is unclear—it seems unlikely that the factors mentioned by Barron (1) for England and Wales (improved antenatal care and general social improvements) are of importance in Sweden during this short period.

A seasonal variation in both spontaneous abortion and stillbirth rates are demonstrated in the present study. An increased rate of spontaneous abortion was found in pregnancies with maternal LMP in June or at the end of the year. These results differ from those from Hungary by Czeizel & Elek (2). They analysed the monthly trends in live births, stillbirths, premature births, spontaneous and induced abortions, as well as conceptions in Hungary in the years 1957–1963. The LMP (last menstrual period) was calculated by counting back for live births and stillbirths nine calendar months, for premature births eight, and for abortions two. The frequency of stillbirths per 1 000 live births showed a seasonal variation with a higher frequency in the cold season, between November and April, than in the warm months between June and September; the corresponding time of conception is Mars–June and August–January respectively. Spontaneous abortion in Hungary did not show any seasonal changes, but the conception of live births fell in January–April and rose to a peak in June–August.

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Table IV *χ^2-analysis of the interaction between two sources of heterogeneity in incidence of stillbirth: maternal age and year of LMP*

Source of heterogeneity	d.f	χ
Total	41	716.0
Between age groups	5	251.9
Between years	6	153.6
Interaction between age groups and years	30	49.5

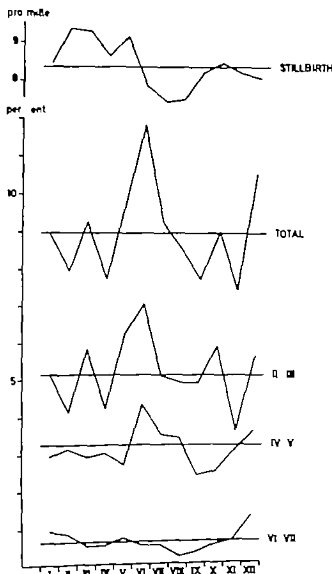


Fig 3 Top graph Stillbirth rate per thousand total births, according to calendar month (Roman numbers on abscissa) Whole of Sweden 1965–1971 Lower graphs Spontaneous abortion rate as percentage of all pregnancies, according to calender month Malmö material 1968–1971 Total=all abortions irrespective of gestational age Roman figures to the right of the graphs show subdivision of material into three subgroups according to gestational age at abortion.

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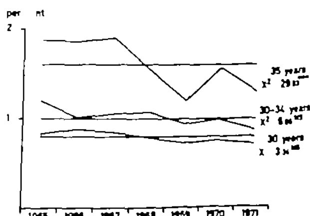


Fig 4 Stillbirth rates by years for three maternal age classes

15. Theander G. Seasonal distribution of births of boys with anomalies of the urethra. Scand J Urol Nephrol 4 1 1970.
16. Le Vann, L. J. Congenital abnormalities in children born in Alberta during 1961: survey and hypothesis. Canad Med Ass J 89 120, 1963.

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We found a corresponding peak for stillbirths around March with a marked decrease during the summer The stillbirth peak corresponds to a low conception rate A similar finding was made by Janerich & Garfinkel (4) in the USA They reported on season of birth and birth order in relation to prenatal pathology from New York records Pre natal pathology—i e late fetal death and/or gross malformation—showed a seasonal variation with peak values during January–February and June The seasonal distribution of births was found to vary between birth orders They did not study the relation based on LMP It is of course im possible to state from such relations that a causative factor gives both a decline in conceptions and a rise in stillbirth rates but this possibility cannot be excluded The rate of spontaneous abortions behaves quite differently The highest incidences are found in pregnancies with LMP in June and December when birth rates are high This could argue for a different causative factor than that connected with stillbirths The two peaks observed agree reasonably well with those published by Mc Donald (7) He reported on a large series from seven hospitals in Quebec Canada during the years 1961–1965 He found a winter excess of abortions (November–April) a pronounced peak in June 1962 and a lesser July–August peak in the other years

Many speculations have been advanced on possible causes of stillbirths and spontaneous abortions which could explain seasonal variations The in fluence of the weather was suggested by Hippocrates and seasonal rhythms in birth of anencephalic infants have been related to various climatic factors such as hours of sunshine (11) and amount of rainfall (16) Among more recent speculations on this theme may be mentioned the possibility of teratogenic alkaloids in blighted potato tubers with a seasonal variation (12)

A report from WHO Scientific Group (14) stated that at present there is no evidence that more widely occurring agents such as pollutants and heavy metals in air or water cause spontaneous abortions not even malnutrition vitamin deficiency or radiation from diagnostic radiography during pregnancy

It is well known however that viral infections can cause spontaneous abortions and fetal death Rubella cytomegalic virus toxoplasma and endocervical infections with mycoplasma organisms are diseases that have this effect Also other infections, such as hepatitis and viral respiratory disease have been associated with increased fetal wastage but the pathogenetic mechanism is less well documented and needs further study (14) As infectious diseases vary in frequency during the year they could ex plain at least part of seasonal rhythms in pregnancy wastage The summer peak of spontaneous abortions, described in the present study is hard to reconcile with an infectious cause—the winter increase better fits that theory Respiratory virus infections, frequent in the beginning of the year could also explain the peak of stillbirths with LMP during that season It is not necessary that the seasonal rhythms observed are due to exogenous influences—they may represent endogenous origins or biological rhythms in reproductive efficiency

REFERENCES

1 Barron S L The epidemiology of human pregnancy Proc Roy Soc Med *61* 1?00 1968

Czeizel E & Elek E. Seasonal changes in the frequency of fetal damages and fertility Gynaecologia *164* 89 1967

3 Ekelund H Kullander S & Källén B Major and minor malformations in newborns and infants up to one year of age Acta Paediat Scand *59* ?97 1970.

4 Janerich D T & Garfinkel J Season of birth and birth order in relation to prenatal pathology Am J Epidemiol 92 351 1970.

5 Kullander S & Källén B Pharmaka und Frauen im Gebärfähigen Alter Bull Schweiz Akad Med Wiss *20* 571 1964

6 Kullander S & Källén B A prospective study of smoking and pregnancy Acta Obstet Gynecol Scand *50* 83 1971

7 McDonald A D Seasonal distribution of abortions. Br J Prev Soc Med *25* 2__ 1971

8 Official Statistics of Sweden Population Changes (SOS) Part 3 National Central Bureau of Statistics. Stockholm. Editions from 1965 to 1971

9 Paukamaa P & Rauha, N Vitamin C deficiency as factor influencing seasonal fluctuation in the frequency of still-births Etudes Neo Nat 6 145 1957

10 Pettersson F Epidemiology of early pregnancy wastage Stockholm 1968

11 Record R G Anencephalus in Scotland Br J Prev Soc Med *15* 93 1961

1 Renwick J H Anencephaly and spina bifida are usually preventable by avoidance of specific but unidentified substance present in certain potato tubers Br J Pre Soc Med *26* 67 197?

13 Slatis H M & de Cloux R. J Seasonal variation in stillbirth frequencies Hum Biol *39* 284 1967

14 Spontaneous and Induced Abortion. WHO Chron *25* 104 1971

Acta Obstet Gynec Scand 53 259–262, 1974

THE RADIO-IMMUNOASSAY OF FOLLICLE STIMULATING HORMONE (FSH) DURING HUMAN PREGNANCY SERUM CONCENTRATION AND RESPONSE TO LUTEINIZING HORMONE RELEASING FACTOR (LRF)

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Abstract Concentrations of follicle-stimulating hormone (FSH) detectable by radio-immunoassay and the FSH response to synthetic luteinizing hormone releasing factor (LRF) ere measured in the sera of fourteen women during the first, the second and the third trimesters of pregnancy Serum FSH was detectable, but relatively low in all the fourteen subjects throughout pregnancy Moreover exogenously administered synthetic LRF failed to stimulate serum FSH secretion from the anterior pituitary and there was no rise in serum FSH in all the fourteen pregnant women Thus, it might be concluded that follicle-stimulating activity of the hypophyseal gland is suppressed throughout human pregnancy by an unknown mechanism

Little is known about the follicle-stimulating activity of the anterior pituitary during human pregnancy The concentrations of follicle-stimulating hormone (FSH) detectable by radio-immunoassay circulating in the blood during human pregnancy were measured using a radio-immunoassay method by Faiman et al (2) and quite high levels of immuno-reactive FSH throughout human pregnancy were reported However in contrast to this report Jaffe et al. (3) observed only low levels of immunoreactive serum FSH early in pregnancy Parlow et al (10) also reported relatively low levels of serum FSH throughout human pregnancy Thus, controversy continued as to the serum concentrations of FSH detectable by radio-immunoassay during human pregnancy

In 1971 the structure of isolated porcine luteinizing hormone releasing factor (LRF) was shown by Schally s group to be (Pyro) Glu-His-Trp-Ser Tyr-Gly Leu-Arg-Pro-Gly NH_2 (1 6 13 14) and the polypeptide corresponding to this structure has been synthesized (7) This synthetic LRF when administered by rapid intravenous or subcutaneous injection, proved to be effective in increasing both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in humans (9 11 14 15) and these observations demonstrated the potential value of LRF in the clinical evaluation of pituitary gonadotrophin secretion

In order to elucidate FSH reserve function of the anterior pituitary during human pregnancy FSH response to synthetic LRF in pregnant women was investigated. The purpose of the present communication is to report the serum concentrations of FSH detectable by radio-immunoassay and the FSH response to synthetic LRF during human pregnancy and to discuss the follicle-stimulating activity of the anterior pituitary during human pregnancy

MATERIALS AND METHODS

Synthetic LRF (MO-1208) was supplied generously by Mochida Pharmaceutical Company Tokyo Japan. The structure of this synthetic LRF is identical with that described by Schally group (1 6, 12, 13). Before initiating this study toxicity studies with single and repeated injections of synthetic LRF were performed in rats to meet requirements of the Ministry of Health and Welfare. No ill effects were observed and all organs were normal macroscopically and histologically

the pituitary "pregnancy cell" remains unknown.

Since human pituitary LH cannot readily be distinguished from human chorionic gonadotrophin (HCG) by routine radio-immunoassay method, only human pituitary FSH is detectable during pregnancy. Faiman et al. (2) reported relatively high levels of FSH detected by radio-immunoassay without variation, throughout human pregnancy. It was reported that serum FSH was detectable in all the forty-five women in concentrations generally in the upper limit of that seen during the normal menstrual cycle and there were no differences in concentration in the trimesters. In contrast to these observations, Jaffe et al. (3) observed decreased FSH levels at the beginning of pregnancy. Parlow et al. (10) also observed relatively low FSH levels throughout pregnancy. According to their report, most FSH values were less than 3 mIU (2nd IRP–HMG)/ml and no value was higher than 6 mIU/ml.

The results of our present communication are in accord with those of the latter two reports. Moreover synthetic LRF failed to stimulate serum FSH secretion from the anterior pituitary in all the fourteen pregnant women given synthetic LRF and no response in serum FSH was demonstrated. Thus, it might be concluded that follicle-stimulating activity of the anterior pituitary is supressed by an unknown mechanism throughout human pregnancy. The anterior pituitary seems to be indispensable in the human only during the first few weeks of pregnancy, in contrast to many other species. It is well known that in humans surgical hypophysectomy as early as the 12th week of gestation is compatible with normal pregnancy and delivery (4, 5).

The question of the concentration and response to LRF of human pituitary LH during pregnancy remains. However, the results of the present communication, namely suppression of pituitary FSH suggest suppression of LH during pregnancy as well.

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REFERENCES

1. Baba, Y., Matsuo, H. & Schally, A. V.: Structure of porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analysis. Biochem Biophys Res Commun *44*: 459, 1971.
2. Faiman, C., Ryan, R. J., Zwirek, S. J. & Rubin, M. E.: Serum FSH and HCG during human pregnancy and puerperium. J Clin Endocrinol *28*: 1323, 1968.
3. Jaffe, R. B., Lee, P. A. & Midgley, A. R.: Serum gonadotropins before, at the inception of and following human pregnancy. J Clin Endocrinol *29*: 1281, 1969.
4. Kaplan, N. M.: Successful pregnancy following hypophysectomy during the twelfth week of gestation. J Clin Endocr *21*: 1139, 1961.
5. Little, B., Smith, O. W., Jessiman, A. G., Selenkow, H. A., Van't Hoff, W., Eglin, J. M. & Moore, F. S.: Hypophysectomy during pregnancy in a patient with cancer of the breast. Case report with hormone studies. J Clin Endocrinol *18*: 425, 1958.
6. Matsuo, H., Baba, Y., Nair, R. M. G., Arimura, A. & Schally, A. V.: Structure of the porcine LH- and FSH-releasing hormone. I. Proposed amino acid sequence. Biochem Biophys Res Commun *43*: 1334, 1971.
7. Matsuo, H., Arimura, A., Nair, R. M. G. & Schally, A. V.: The synthesis of porcine LH- and FSH-releasing hormone by the solid phase method. Biochem Biophys Res Commun *45*: 654, 1971.
8. Midgley, A. R., Jr.: Radioimmunoassay of human follicle-stimulating hormone. J Clin Endocrinol *27*: 295, 1967.
9. Nakano, R., Kotsuji, F., Mizuno, T., Hashiba, N., Washio, M. & Tojo, S.: Response to luteinizing hormone releasing factor (LRF) in normal subjects and anovulatory patients. Acta Obstet Gynecol Scand *52*: 171, 1973.
10. Parlow, A. F., Daane, T. A. & Dignam, W. J.: On the concentration of radioimmunoassayable FSH circulating in blood throughout human pregnancy. J Clin Endocrinol *31*: 213, 1970.
11. Rebar, R., Yen, S. S. C., Vandenberg, G., Naftolin, F., Ehara, Y., Engblom, S., Ryan, K. J., Rivier, J., Amoss, M. & Guillemin, R.: Gonadotropin responses to synthetic LRF: Dose–response relationship in men. J Clin Endocrinol *36*: 10, 1973.
12. Schally, A. V., Arimura, A., Baba, Y., Nair, R. M. G., Matsuo, H., Redding, T. W. & Debeljuk, L.: Isolation and properties of the FSH- and LH-releasing hormone. Biochem Biophys Res Commun *43*: 393, 1971.
13. Schally, A. V., Baba, Y., Arimura, A., Redding, T. W. & White, W. F.: Evidence for peptide nature of LH- and FSH-releasing hormones. Biochem Biophys Res Commun *42*: 50, 1971.

Table I *Effect of LRF on the serum concentration of FSH in pregnant women in the first trimester*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of FSH in mIU 2nd IRP HMG/ml serum					
		0	15	30	45	60	120
Subject	Week of pregnancy						
Y S	6	4.8	5 6	5.3	7 2	7 7	5 9
M H	6	8.3	8 2	9 4	8.3	8 9	8.2
Y T	8	6 0	6 6	6 9	5 1	5 6	5.8
I H	9	4 7	5 4	6.8	6 4	7.8	7.8
T N	10	7.5	7.5	7.5	8 0	8.5	7.5

Volunteers

Fourteen normal pregnant volunteers were given synthetic LRF Subjects ranged in age from 22 to 34 years and laboratory tests gave normal results Five volunteers were in the first trimester of pregnancy five were in the second and four were in the third trimester of pregnancy Volunteers in the first and the second trimester of pregnancy were scheduled to have abortion induced on socio-medical indications according to Japanese law Consent was obtained from each subject after full explanations of the purpose and nature of all procedures of this study

LRF administration

Synthetic LRF was administered intravenously over 30 sec in a dose of 200 μg to each subject Blood was sampled at zero time and at 15 30 45 60 120 min for determination of serum FSH concentration Immunoreactive serum FSH levels were assayed in duplicate by double antibody radio-immunoassay according to the method of Midgley (8) with minor modifications Both the second international reference preparation of human menopausal gonadotrophin (2nd IRP-HMG) and LER 907 were used to obtain standard curves The average relative potencies of these preparations were 49 IU of 2nd IRP-HMG/mg LER 907 for FSH The results of this study were expressed as mIU of 2nd IRP-HMG/ml serum

Table II *Effect of LRF on the serum concentration of FSH in pregnant women in the second trimester*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of FSH in mIU 2nd IRP-HMG/ml serum					
		0	15	30	45	60	120
Subject	Week of pregnancy						
N Y	19	2.3	2.3	2.5	[illegible]	2.8	2.8
S H	20	2.8	2.1	[illegible]	2 4	2 9	2 3
Y H	22	5 6	4 4	4.8	5 1	6.2	4.5
Y T	23	3 0	4.2	2.6	2 6	2.8	–
S M	27	[illegible]	3	2.2	2.8	4.8	2.5

Table III *Effect of LRF on the serum concentration of FSH in pregnant women in the third trimester*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of FSH in mIU 2nd IRP-HMG/ml serum					
		0	15	30	45	60	120
Subject	Week of pregnancy						
A T	34	3.6	4 9	3 7	4 1	[illegible]	2.9
K Y	34	3 0	2.7	3 6	2.7	3.2	3 0
K K.	36	3 8	3 6	2 8	5 9	3.2	3 [illegible]
Y K	38	2.2	2.5	[illegible]	2.6	3.6	3 [illegible]

RESULTS

The serum concentration of FSH before and after the intravenous injection of 200 μg of LRF in fourteen subjects in the first the second and the third trimester of pregnancy are shown in Tables I II and III

Serum FSH was detectable in all the fourteen subjects but FSH levels were relatively low throughout pregnancy Most values were less than 5 mIU/ml and no value was higher than 8 3 mIU/ml These FSH concentrations were lower than the lowest level normally seen in the menstrual cycle (Tables I II and III)

All the fourteen subjects in the first the second and the third trimester of pregnancy showed no response to the exogenous administration of synthetic LRF and there was no rise in serum FSH after LRF injection (Tables I II and III)

No serious side effects (change in blood pressure, pulse or respiration) were noted in any of the subjects given synthetic LRF and the course of pregnancy after LRF administration was uneventful

DISCUSSION

The gonadotrophic function of the anterior pituitary during human pregnancy is still not known and remains open to speculation There is ample evidence that the pituitary gland increases in weight and undergoes histological alterations during pregnancy but the genesis and physiological role of

the pituitary "pregnancy cell" remains unknown

Since human pituitary LH cannot readily be distinguished from human chorionic gonadotrophin (HCG) by routine radio-immunoassay method only human pituitary FSH is detectable during pregnancy. Faiman et al. (2) reported relatively high levels of FSH detected by radio-immunoassay without variation throughout human pregnancy. It was reported that serum FSH was detectable in all the forty-five women in concentrations generally in the upper limit of that seen during the normal menstrual cycle and there were no differences in ncentration in the trimesters. In contrast to these servations Jaffe et al. (3) observed decreased SH levels at the beginning of pregnancy. Parlow al. (10) also observed relatively low FSH levels roughout pregnancy. According to their report, ost FSH values were less than 3 mIU (2nd IRP-MG)/ml and no value was higher than 6 IU/ml.

The results of our present communication are accord with those of the latter two reports. loreover synthetic LRF failed to stimulate serum SH secretion from the anterior pituitary in all e fourteen pregnant women given synthetic LRF nd no response in serum FSH was demonstrated hus, it might be concluded that follicle-stimulat g activity of the anterior pituitary is supressed y an unknown mechanism throughout human regnancy. The anterior pituitary seems to be indispensable in the human only during the first few eeks of pregnancy in contrast to many other pecies. It is well known that in humans surgical hypophysectomy as early as the 12th week of gestation is compatible with normal pregnancy and delivery (4, 5).

The question of the concentration and response to LRF of human pituitary LH during pregnancy remains. However the result of the present communication, namely suppression of pituitary FSH suggest suppression of LH during pregnancy as well.

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REFERENCES

1. Baba, Y. Matsuo H. & Schally A. V. Structure of porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analysis. Biochem Biophys Res Commun 44 459 1971
2. Faiman, C. Ryan, R. J. Zwirek, S. J. & Rubin, M. E. Serum FSH and HCG during human pregnancy and puerperium. J Clin Endocrinol 28 1323 1968.
3. Jaffe, R. B. Lee, P. A. & Midgley A. R. Serum gonadotropins before at the inception of and following human pregnancy. J Clin Endocrinol 29 1281 1969
4. Kaplan, N. M. Successful pregnancy following hypophysectomy during the twelfth week of gestation. J Clin Endocr 21 1139 1961
5. Little B. Smith, O. W. Jessiman, A. G. Selenkow H. A., Van't Hoff W. Eglin J. M. & Moore, F. S.. Hypophysectomy during pregnancy in patient with cancer of the breast. Case report with hormone studies. J Clin Endocrinol 18 425 1958
6. Matsuo, H. Baba, Y. Nair R. M. G. Arimura, A. & Schally A. V. Structure of the porcine LH- and FSH-releasing hormone. I. Proposed amino acid sequence. Biochem Biophys Res Commun 43 1334 1971
7. Matsuo H. Arimura, A. Nair R. M. G. & Schally A. V. The synthesis of porcine LH and FSH-releasing hormone by the solid phase method. Biochem Biophys Res Commun 45 654 1971
8. Midgley A. R., Jr. Radioimmunoassay of human follicle-stimulating hormone. J Clin Endocrinol 27 295 1967
9. Nakano, R., Kotsuji, F. Mizuno T. Hashiba, N. Washio M. & Tojo S. Response to luteinizing hormone releasing factor (LRF) in normal subjects and anovulatory patients. Acta Obstet Gynecol Scand 52 171 1973
10. Parlow A. F. Daane T. A. & Dignam, W. J. On the concentration of radioimmunoassayable FSH circulating in blood throughout human pregnancy. J Clin Endocrinol 31 213 1970
11. Rebar R., Yen, S. S. C. Vandenberg, G. Naftolin, F. Ehara, Y. Engblom, S., Ryan, K. J. Rivier J. Amoss, M. & Guillemin, R.. Gonadotropin responses to synthetic LRF. Dose-response relationship in men. J Clin Endocrinol 36 10, 1973
12. Schally A. V. Arimura, A. Baba, Y. NAir R. M. G. Matsuo H. Redding, T. W. & Debeljuk, L. Isolation and properties of the FSH and LH-releasing hormone. Biochem Biophys Res Commun 43 393 1971
13. Schally A. V. Baba, Y. Arimura, A. Redding, T. W. & White, W. F. Evidence for peptide nature of LH- and FSH-releasing hormones. Biochem Biophys Res Commun 42 50, 1971

14 Schally A V Kastin A J & Arimura, A Hypothalamic FSH and LH-regulating hormone Structure, physiology and clinical studies Fertil Steril *22* 703 1971
15 Yen S S C Rebar R Van den Berg, G Naftolin F Ehara, Y Engblom S Ryan, K. J Benirschke K Rivier J Amoss M & Guillemin R Synthetic luteinizing hormone releasing factor A potent stimulator of gonadotropin release in man J Clin Endocrinol *34* 1108 1972

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TRANSABDOMINAL ISTHMIC AMNIOCENTESIS IN RH IMMUNIZATION WITH PARTICULAR REFERENCE TO COMPLICATIONS

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Abstract A retrospective examination has been made of 530 transabdominal isthmic amniocenteses performed on 231 Rh-immunized women in the period from 1969 to 1973. Technique and complications are described. This study indicates the advantage of a transisthmic approach which offers a minimum risk for the fetus and a greater success in carrying out the procedure.

Amniocentesis has up to now most frequently been conducted in the examination of the Rh-immunized woman. However there are other conditions of pregnancy that call for this procedure intrauterine asphyxia, assessment of maturity and antenatal diagnosis of genetic and metabolic disorders.

The complications that can be encountered with this procedure are lesions of the fetus, placenta, or umbilical cord with the consequent risk of bleeding and intrauterine fetal death (1 3 4 5 10 11 12).

The preponderance of publications indicate that amniocentesis is performed transabdominally through the corpus uteri (5 6, 7 10 12 14) below the preabdominal amniocentesis with needle insertion through the isthmus uteri (8 13 15) below the presenting part of the fetus has been used routinely since 1963 by the staff in the Department of Obstetrics and Gynaecology Rikshospitalet, Oslo A retrospective examination has been made of the results obtained for the period from 1969 to 1973 during which 530 amniocenteses were performed on 231 Rh-immunized women

TECHNIQUE

The bladder is voluntarily emptied before the procedure. The abdominal skin is prepared by the usual preoperative methods. Local anaesthesia is unnecessary. The presenting part is pushed up so that fingers may be inserted underneath for guidance and protection. The needle is inserted in the midline just under the presenting part approximately 2 or 3 fingers above the symphysis. If the patient has great anxiety or the presenting part is engaged in the pelvic inlet, diazepam or pethidine may be used. If necessary the presenting part can be pushed up from the vagina by an assistant. Fetal heart sounds are always checked before and after the procedure.

RESULTS

Table I shows the number of amniocenteses in relation to duration of pregnancy Table II shows the distribution of the number of amniocenteses performed in Liley s zones I II and III Table III presents the number of unsuccessful amniocenteses in relation to duration of pregnancy Amniocentesis is considered successful when the amniotic fluid quantitatively and qualitatively is suitable for spectrophotometry The procedure was not successful in 20 cases (3.8 %) in one case the presenting part could not be moved into position above the pelvic inlet.

Failure of transisthmic amniocentesis was, in the majority of cases due to oligohydramnios or a complete breech presentation. In one case of failure an oedematous placenta was localized in the lower anterior wall Rupture of the membranes occurred within one or within two days of amniocentesis in three and two cases respectively Spontaneous premature labour occurred within one day in one case. In all the cases in which membrane rupture or labour developed the pregnancy was more advanced than 239 days Placental abruption oc

14 Schally A V Kastin, A J & Arimura A Hypothalamic FSH and LH-regulating hormone Structure physiology and clinical studies Fertil Steril 22 703 1971
15 Yen S S C Rebar R Van den Berg, G Naftolin F Ehara, Y Engblom, S Ryan K J Benirschke K Rivier J Amoss M & Guillemin R Synthetic luteinizing hormone releasing factor A potent stimulator of gonadotropin release in man J Clin Endocrinol 34 1108 1972

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2. Queenan, J T & Adams, D W Obstet Gynecol 24 530, 1964
3. Strand, A.: I Intrauterine Dangers to the Foetus (ed. J Horsky and Z. K. Stembera), p. 390 Excerpta Medica Foundation, Amsterdam, 1967
4. Walker A H C. & Jennison R. F Br Med J 2 1152, 1962.
5. Wiklund, D.: Acta Obstet Gynecol Scand 48 Suppl 3 147 1969
6. Woo Wang, M Y F McCutcheon, E. & Desforges, J F Amer J Obstet Gynecol 97 1123 1967

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Table I *Number of amniocentesis in relation to duration of pregnancy*

Duration of pregnancy (days)	Number
<210	147
211–238	126
239–266	218
>267	39
Total	530

Table II *Distribution of the number of amniocenteses performed in the Liley zones I II and III*

Zone	Number
I	127
II	317
III	65
Total	509

curred within a few hours of amniocentesis in one case and the child was successfully delivered by caesarean section No other complications were recorded

DISCUSSION

A retrospective examination has been made of 530 amniocenteses performed on 231 Rh-immunized women in the period from 1969 to 1973 Pertinent to this study are the results reported in other publications (1 2 5 12, 13 14 15 16) and in Table IV they are grouped according to method employed (A) Insertion through corpus uteri (B) Insertion through the isthmus uteri and (C) Ultrasonic location of fetus placenta to aid insertion of needle

It is readily observed that by comparison of A and B there are fewer failures when trans

Table III *Number of unsuccessful amniocenteses in relation to duration of pregnancy*

Duration of pregnancy (days)	Number
<210	13
211 238	5
239–266	2
>267	0
Total	20

Table IV *Tabulation of results reported in* [illegible] *publications grouped according to method em*[illegible] *ployed*

Authors	Number of punctures	Failures (%)
A. Walker & Jennison	156	5
Fairweather & Walker	187	8.5
Queenan & Adams	74	5
Alpern et al	101	8
Woo Wang et al	89	11.2
B Strand	370	[illegible]2
Wiklund	118	0.8
Ekgren & Moe	530	3.8
C. Bang & Northeved*	68	5.9

A new ultrasonic method for transabdominal amnioc[illegible]tesis was used

abdominal isthmic amniocentesis is performed. T[illegible] additional advantage of this approach is the d[illegible] creased chance of puncturing the fetus placen[illegible] or umbilical cord In the few cases where placen[illegible] lesions do occur with this method they result fr[illegible] the placenta being located anteriorly on the lo[illegible] uterine segment Furthermore the transisthmic i[illegible]sertion does not appear to increase the chance [illegible] premature labour membrane rupture or lab[illegible] following amniocentesis occurred no earlier th[illegible] the 239th day of pregnancy and had no influen[illegible] on fetal morbidity or mortality

The present study indicates the advantage [illegible] transabdominal isthmic amniocentesis It offer[illegible] minimum risk for the fetus and is rarely unsucc[illegible]ful

REFERENCES

1 Alpern W M Charles A G & Friedman, E. Amer J Obstet Gynecol *95* 11.3 1966.
2 Bang J & Northeved A Amer J Obstet Gyne[illegible] *114* 599 1972
3 Berner H W Facog Seisler E P & Barlow [illegible] Obstet Gynecol *40* 599 1972
4 Crystle C D & Rigsby W C Amer J Ob[illegible] Gynecol *106* 310 1970
5 Fairweather D V I & Walker W J Ob[illegible] Gynaecol Br Comm *71* 48, 1964
6 Fort A T Amer J Obstet Gynecol *110* [illegible] 1971
7 Freda V J Amer J Obstet Gynecol *92* 341 [illegible]
8. Haworth S G Milic A B & Adamsons K. [illegible] Obstet Gynecol *11* 1182 1968.
9 Liley A W New Z Med J *59* 581 1960
10 Little B McCutcheon E. & Desforges, J F N[illegible] Engl Med J *274* 332 1966.
11 Misenheimer H R Amer J Obstet Gynecol [illegible] 1133 1966.

Acta Obstet Gynec Scand 53 267–269 1974

EFFECT OF ESTROGEN THERAPY ON CLIMACTERIC SYMPTOMS AND TISSUE CHANGES

Reijo Punnonen and Lauri Rauramo

From the Department of Obstetrics and Gynecology (Head Professor Lauri Rauramo) University Central Hospital, Turku Finland

Abstract. Estrogen substitution for 50 castrated women consisted of estriol succinate and for another 50 women of estradiol valerate. The daily dose in both groups was 2 mg, given for six months starting one month after castration. The patients generally developed strong vegetative symptoms after oophorectomy. Five patients failed to respond to estriol succinate and one to estradiol valerate. The effect of estrogen therapy on the vaginal smear and the skin was also studied in these six cases. An estrogen effect, though often fairly weak, in the vaginal smear was observed. Total urinary estrogens were high in all the cases after three and six months of estrogen therapy. The epidermis was significantly thickened after three months of estrogen except in one case. There was generally no significant difference between the 3-month and 6-month skin specimens. Ostensibly ineffective estrogen substitution may thus play a role in the prevention of postmenopausal degenerative changes. The effect of estrogen therapy on the vegetative symptoms alone should not be regarded as the yardstick of its efficacy.

Our knowledge of the physiology and biochemistry of hormones is based for the most part on test animal studies. Large doses of hormones have generally been used and the reactions provoked cannot always be regarded as physiological effects. In addition it is necessary to consider the different ways in which animal species react to a hormone or drug (Diczfalusy & Lauritzen 1961). Despite the enormous increase in the use of estrogens in recent years little is known even about the actions of these hormones in man. The climacteric constitutes an important time for estrogen therapy. Estrogens generally have a very beneficial effect on typical climacteric symptoms such as sweating and hot flushes. The symptoms usually disappear or abate after a few weeks of therapy. Estrogen therapy has been tried for the prevention and retardation also of many postmenopausal degenerative changes. However, sometimes even long-term or intensive estrogen therapy fails to eliminate the vegetative symptoms. The aim of our study was to analyse such cases in more detail. Is there possibly some kind of disturbance in the function of estrogen receptors in the cells or are the estrogen-induced tissue changes also developing normally in these patients?

MATERIAL AND METHODS

The patients were castrated one month before the institution of estrogen therapy. Oophorectomy was performed in conjunction with hysterectomy undertaken for different reasons. Pronounced climacteric symptoms, especially sweating and hot flushes, generally appeared soon after the operation. Substitution therapy consisted of estriol succinate in 50 and estradiol valerate in 50 patients. The daily dose for both groups was 2 mg. Therapy was started one month after castration and was continued for six months. The mean age of the patients treated with estriol succinate was 49 and that of the women managed with estradiol valerate 48 years. The six-month course had no effect on sweating and hot flushes in five cases of the estriol succinate and one patient of the estradiol valerate group. The serum sodium of all these patients was within normal limits. In addition to the effect on vegetative symptoms, the skin effect of estrogen therapy was also studied. Skin biopsies were taken from the lateral aspect of the thigh about 15 cm above the femoral epicondyle before the institution of therapy and after three and six months estrogen therapy. It was mainly the thickness of the epidermis that was studied, using planimetry for the evaluation. The method of taking the skin specimens and the planimetry were described earlier (Rauramo and Punnonen, 1969 and 1971; Punnonen, 1972). Along with the skin studies, vaginal smear examinations were made (Papanicolaou 1933, Frost et al. 1955) and total urinary estrogens were also determined.

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Table I *Cases in which 6-month estrogen therapy had no effect on the vegetative symptoms*

The first five patients were managed with estriol succinate the sixth with estradiol valerate

Patient	Age	Planimetric measurements		Total estrogens (μg/24 h)	Maturation index
1	48	1	40.5	22	0/98/2
		2	50 6	268	0/93/7
		3	50 0	260	0/95/5
2	48	1	43.5	6 9	0/97/3
		2	56 1	177 0	0/98/2
		3	54 3	105 0	0/97/3
3	47	1	45 8	20 8	100/0/0
		2	47 1	136 0	1/95/4
		3	46 1	43.5	–
4	50	1	45 1	19 1	4/83/13
		2	49 1	235.5	0/67/33
		3	44.2	228 0	–
5	47	1	37 0	–	48/50/2
		2	47.2	–	6/88/6
		3	48 0	–	35/67/8
6	52	1	40 9	23 1	45/52/3
		2	54 4	400 0	0/97/3
		3	42.3	153 0	0/90/10

RESULTS

Table I shows the planimetric measurements of the thickness of the epidermis before the institution of estrogen treatment (1) and after three (2) and six (3) months of therapy The maturation indices for the same periods and the total urinary estrogen values are also presented The first five patients of the table were given estriol succinate and the sixth received estradiol valerate (Table I) The vaginal smears displayed such marked inflammation in two cases after six months of estrogen therapy that cytohormonal study was not possible The urinary estrogens of one patient were not studied

DISCUSSION

The causes of vegetative symptoms in the climacteric are not fully known They are mostly associated with a lowered estrogen level Lowering of the estrogen level probably causes a hypothalamic disturbance resulting in disruption of the quilibrium of the autonomic nervous system Estrogens must in fact be regarded as stabilisers of the autonomic nervous system in women (Hauser 1960) Sweating and hot flushes are often particularly intense if oophorectomy is performed on a woman who is still menstruating On the other hand, if castration is carried out at an early age these vegetative symptoms hardly ever occur An explanation offered for this is the greater ability of the adrenals at an early age to compensate for the missing ovarian function However these patients sometimes develop typical climacteric symptoms at an age when the menopause would have occured normally Estrogen therapy generally exerts a beneficial action on sweating symptoms. Six-months estrogen therapy failed to produce any effect on the vegetative symptoms of six of the 100 castrated women in our study The therapy was estriol succinate in five and estradiol valerate in one of these cases. The total urinary estrogen level was high in all the cases after both three- and six mnonth therapy There are obviously other causative agents than mere lack of estrogens that contribute to the origin of vegetative symptoms Ageing changes of the hypothalamus and disturbances of its function are probably the causative agents to be considered primarily

Examination of the effect of estriol succinate therapy on the skin revealed statistically significant ($p<0.01$) thickening of the epidermis in three out five cases after three months of treatment There was no statistically significant difference in epidermal thickness between the 3-month and 6-month specimens The epidermis was significantly thickened after three months treatment in one case. However no statistically significant difference was seen between the pre therapeutic and six-month specimens There was only one case in which neither three nor six months estriol succinate has any effect on the thickness of the epidermis W S Bullough (1955) found that estriol exerted a stronger mitogenic effect on mouse epidermis in vitro than estradiol and estrone Demetriou (1964) proved the sensitivity of skin to estrogens to be fully comparable with that of the so-called target organs We have also reported results showing the favourable action of estriol on the skin of castrated women (Rauramo & Punnonen 1969 Punnonen 1972) The daily dose of long-term estrogen therapy need not be very high to achieve a beneficial skin effect The estrogen effect in the vaginal smears of our patients was usually weak after both three and six months therapy in spite of the high urinary estrogen values Vaginal epithelium develops a tolerance to a constant dose in long-term estrogen therapy Epithelial growth is produced only by increasing the dose (Nieburgs 1958)

It seems evident from our study that ostensibly ineffective estrogen therapy, i.e. treatment to which the patient's subjective symptoms fail to respond, may have a beneficial effect on postmenopausal metabolic changes. Estrogen substitution may in itself play a role in preventing postmenopausal degenerative changes.

REFERENCES

1. Bullough, W. S. Hormones and mitotic activity. Vitamins Hormones *11*: 261, 1953.
2. Demetriou, J. A., Crowley, L. G., Kushinsky, S., Donovan, A. J., Kotin, P. & Macdonald, I. Radioactive estrogens in tissues of postmenopausal women with breast neoplasms. Cancer Res *24*: 926, 1964.
3. Diczfalusy, E. & Lauritzen, C. Oestrogene beim Menschen. Springer Verlag, Berlin, Göttingen and Heidelberg, 1961.
4. Frost, P., Wood, D. A. & Kearney, J. V. Vaginal smear hormonal patterns in adenocarcinoma of the breast. In Intersociety Cytology Council Transactions, p. 93. San Francisco, 1953.
5. Hauser, G. A. Die Rolle des neurovegetativen Nervensystems in der Gynäkologie und Geburtshilf. Fortschr Geburtsh Gynakol *10*: 17, 1960.
6. Nieburgs, H. E. Cytolysis and long-term administration estrogens. Acta Cytol *2*: 370, 1958.
7. Papanicolaou, G. N. The modifying effect of old age upon the oestrous cycle in the guinea pig. Anat Rec *55*: Suppl. 4, 71, 1933.
8. Punnonen, R. Effect of castration and peroral estrogen therapy on the skin. Acta Obstet Gynecol Scand Suppl. 21, 1972.
9. Rauramo, L. & Punnonen, R. Wirkung einer oralen Östrogentherapie mit Östriolsuccinat auf die Haut kastrierter Frauen. Z Haut Geschlechtskr *44*: 13, 463, 1969.
10. Rauramo, L. & Punnonen, R. Über die Wirkung per oraler Oestrogentherapie mit Oestradiolvalerat auf die Haut kastrierter Frauen. Arch Gynakol *201*: 202, 1971.

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		3	54.3	105 0	0/97/3
3	47	1	45.8	70 8	100/0/0
		2	47 1	136.0	1/95/4
		3	46.1	43.5	–
4	50	1	45 1	19 1	4/83/13
		2	49 1	235.5	0/67/33
		3	44 2	228 0	–
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Table I shows the planimetric measurements of the thickness of the epidermis before the institution of estrogen treatment (1) and after three (2) and six (3) months of therapy The maturation indices for the same periods and the total urinary estrogen values are also presented The first five patients of the table were given estriol succinate and the sixth received estradiol valerate (Table I) The vaginal smears displayed such marked inflammation in two cases after six months of estrogen therapy that cytohormonal study was not possible The urinary estrogens of one patient were not studied

DISCUSSION

The causes of vegetative symptoms in the climacteric are not fully known They are mostly associated with a lowered estrogen level Lowering of the estrogen level probably causes a hypothalamic disturbance resulting in disruption of the quilibrium of the autonomic nervous system Estrogens must in fact be regarded as stabilisers of the autonomic nervous system in women (Hauser 1960) Sweating and hot flushes are often particularly intense if oophorectomy is performed on a woman who is still menstruating. On the other hand if castration is carried out at an early age these vegetative symptoms hardly ever occur An explanation offered for this is the greater ability of the adrenals at an early age to compensate for the missing ovarian function However these patients sometimes develop typical climacteric symptoms at an age when the menopause would have occured normally Estrogen therapy generally exerts a beneficial action on sweating symptoms Six-months estrogen therapy failed to produce any effect on the vegetative symptoms of six of the 100 castrated women in our study The therapy was estriol succinate in five and estradiol valerate in one of these cases The total urinary estrogen level was high in all the cases after both three- and six-mnonth therapy There are obviously other causative agents than mere lack of estrogens that contribute to the origin of vegetative symptoms Ageing changes of the hypothalamus and disturbances of its function are probably the causative agents to be considered primarily

Examination of the effect of estriol succinate therapy on the skin revealed statistically significant ($p<0.01$) thickening of the epidermis in three out of five cases after three months of treatment. There was no statistically significant difference in epidermal thickness between the 3-month and 6-month specimens The epidermis was significantly thickened after three months treatment in one case However no statistically significant difference was seen between the pre-therapeutic and six-month specimens There was only one case in which neither three nor six months estriol succinate had any effect on the thickness of the epidermis. W S Bullough (1955) found that estriol exerted a stronger mitogenic effect on mouse epidermis in vitro than estradiol and estrone Demetriou (1961) proved the sensitivity of skin to estrogens to be fully comparable with that of the so-called target organs We have also reported results showing the favourable action of estriol on the skin of castrated women (Rauramo & Punnonen 1969 Punnonen 1972) The daily dose of long-term estrogen therapy need not be very high to achieve a beneficial skin effect The estrogen effect in the vaginal smears of our patients was usually weak after both three and six months therapy in spite of the high urinary estrogen values Vaginal epithelium develops a tolerance to a constant dose in long-term estrogen therapy Epithelial growth is produced only by increasing the dose (Nieburgs 1958)

LABILITY OF HUMAN DECIDUAL CELLS IN VIVO EFFECTS OF HYPERTONIC SALINE

Björn Gustavii and Ulf Brunk

From the Department of Obstetrics and Gynecology (Head: Prof. Lars Philip Bengtsson), University Hospital, Lund and the Institute of Pathology (Head: Prof. Jan Pontén), University of Uppsala, Sweden

Abstract. Recently decidual cells, in contrast to trophoblastic cells, were found to be exceedingly fragile in vitro. In the present investigation the stability in vivo, of decidual cells was studied by histological and cytochemical methods. The material was obtained either by vacuum aspiration or hysterotomy at various intervals following intrauterine injection of 20 % saline solution. Fifteen minutes following the injection, decidual cells in part of the decidua showed distinct degenerative changes and signs of leakage into the cell cytoplasm of the lysosomal marker enzyme acid phosphatase. The extent of these changes and the number of cells involved increased in parallel with the interval between the injection and the evacuation. The alterations in the decidual cells were of the same type and magnitude whether the hypertonic solution was injected intra-amniotically or extra-amniotically. The present findings further support the hypothesis that the mechanism by which hypertonic saline provokes abortion involves damage of the decidua and subsequent liberation of prostaglandins.

The mechanism by which hypertonic saline injected into the uterine cavity induces abortion is still uncertain. In our previous works attention was focused on the possible role played by the decidua in this context (3, 9–13). Thus pronounced degenerative changes were found in the placental decidua following both intra-amniotic and extra-amniotic injection of hypertonic saline, while the major part of the trophoblast was microscopically intact (12). It was also found that extra-amniotic injection of hypertonic saline was followed by the appearance of prostaglandin $F_{2\alpha}$ in the amniotic fluid (13). This compound, which has a pronounced contractile effect on the myometrium, has been demonstrated by Karim & Devlin (15) to be present in high concentration in the decidua during labor.

We have also found that decidual cells, in contrast to trophoblastic cells, undergo rapid autolysis in vitro and are extremely sensitive to low effective osmotic pressure during fixation (3). The present study reports the results of a histological and cytochemical investigation on the stability in vivo of decidual cells obtained at various intervals following an intrauterine injection of a 20 % saline solution.

MATERIAL AND METHODS

Fifteen physically healthy women were subjected to therapeutic abortion by either vacuum aspiration (11th–12th week of pregnancy) (6 cases), hysterotomy (15th–20th week of pregnancy) (6 cases), or intrauterine injection of hypertonic saline (15th–20th week of pregnancy) (3 cases). As described in detail below the women undergoing vacuum aspiration or hysterotomy also received an intrauterine injection of hypertonic saline before operation. In the material obtained, the examination was confined to the decidua parietalis. No attempt was made to evaluate the changes in the decidua basalis in the basal plate of the placenta due to the normally occurring regressive changes in the decidual cells adjacent to Nitabuch membrane.

Material obtained from aspiration abortion

Fifty to one hundred ml of 20 % sodium chloride solution (~6 600 mOsm) was injected extra-amniotically as previously described (8). Vacuum aspiration was performed either 15 minutes (3 cases) or 60 minutes (3 cases) following the injection. The aspirated material, obtained within 60 seconds of the beginning of the aspiration was collected in glass bottle kept on ice and containing fixative solution consisting of 3 % formaldehyde (obtained from paraformaldehyde) in 0.15 M Na-cacodylate-HCl buffer pH 7.4 (~1350 mOsm). The fixation was performed at 0–4°C for 12–18 hours.

Material obtained by hysterotomy

One hundred and fifty to two hundred ml of a 20 % sodium chloride solution was injected intra-amniotically (3 cases) as previously described (1), or extra-amniot-

BOOKS RECEIVED

Urodynamics Upper and Lower Urinary Tract Editors Lutzeyer W and Melchior H Technische Hochschule Aachen 209 figs XII 344 pages 1973 Cloth DM 128 – US $52.50 Berlin–Heidelberg New York Springer Verlag.

The book stands for our present knowledge of urodynamics It is recommended to all gynecologists, dealing with gynecological urology

I S.

Fig. 4. Cytochemical demonstration of acid phosphatase in normal decidual cells obtained by vacuum aspiration. A generally granular distribution of reaction product. Incubation time 90 minutes. × 1200.

Demonstration of acid phosphatase. Fifteen minutes following the injection decidual cells in part of the decidua showed diffuse cytoplasmic staining and only few granular precipitates of reaction products, indicating redistribution of enzymes from their initial lysosomal sites as a result of damage to the membranes (compare Figs. 4 and 5). In the material obtained 60 minutes or 3 hours after the injection, quite a number of decidual cells still showed a granular reaction pattern. Not until after abortion were all decidual cells diffusely stained.

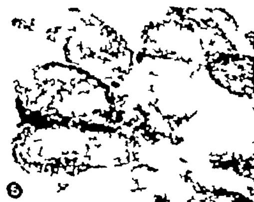


Fig. 5. Cytochemical demonstration of acid phosphatase in decidual cells exposed to hypertonic saline injected extra-amniotically 15 minutes before vacuum aspiration. Diffuse cytoplasmic staining indicating diffusion of hydrolytic enzymes from their initial lysosomal sites into the cell cytoplasm. Note differences in degree of staining between individual cells. Only scanty granules can be seen. Incubation time 90 minutes. × 1200.

DISCUSSION

It has been demonstrated previously that hypertonic saline does not induce abortion by damaging the tissues within the amniotic sac or the fetal portion of the placenta but rather by acting on extra-amniotic tissues (10). It was suggested that the decidua, which lies outside the amniotic sac might be the target for the action of hypertonic saline. Our observation that 20 % saline induced decidual changes of the same appearance whether injected intra-amniotically or extra-amniotically are in agreement with this assumption. The intra-amniotically injected saline reaches the decidual tissue apparently by diffusion through the fetal membranes. The findings also seem to explain why the interval between injection and abortion is approximately equal in both methods of injection (2, 14).

Our observation that hypertonic saline rapidly damages decidual cells in vivo corroborates the findings of a previous in vitro study showing that decidual cells are exceedingly sensitive to various kinds of noxious stimuli (4). This fragility of decidual cells might explain why even isotonic saline when injected extra-amniotically can induce abortion (10).

In the present study cytochemical methods were used for demonstrating the lysosomal marker enzyme acid phosphatase (7). It was found that hypertonic saline induced leakage of the enzyme into the cell cytoplasm. Thus fifteen minutes following the injection, decidual cells in part of the decidua showed diffuse cytoplasmic staining and only few granular precipitates of reaction products. The longer the interval between the injection of the hypertonic saline and the evacuation of the uterine contents, the larger the number of cells with signs of enzyme diffusion. In the material obtained after abortion, all decidual cells were diffusely stained.

Diffusion of hydrolytic enzymes from the lysosomes would result in cellular degradation since together these enzymes are capable of breaking down most or all cellular constituents (5). It seems very probable that the enzyme after diffusion into the cytoplasm cleave prostaglandin precursors from membrane phospholipids, and by this action provide substrate for prostaglandin synthesis. Therefore the stability of lysosomes in decidual cells might be of major significance in the maintenance of pregnancy.

It has been shown previously that (*a*) prostaglan-



Fig. 1. Noraml decidual cells obtained by vacuum aspiration. H E ×485

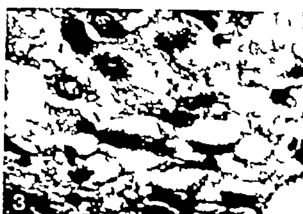


Fig. 3. Decidual cells obtained by vacuum aspiration from a woman who had aborted 48 hours following extra-amniotic injection of hypertonic saline. The cells show extensive degeneration with ballooning, vacuolization of the cytoplasm and pyknotic or disintegrating nuclei. H E ×485

cally (3 cases) (8). Evacuation of the conceptus and removal of the parietal decidua was performed 3 hours after the injection by hysterotomy including curettage of the uterine wall. The decidual tissue obtained was immediately placed in the above fixative and kept at 0–4°C for 12–18 hours.

Material obtained by aspiration curettage after the abortion

One hundred and fifty to two hundred ml of a 20 % sodium chloride solution was injected extra-amniotically (3 cases) (8). Immediately after abortion, which occurred 5–48 hours following the injection, the remaining contents of the uterine cavity were removed by vacuum aspiration. The aspirated material was collected and fixed as described above.

Control material

Untreated decidua, obtained either by vacuum aspiration or by hysterotomy, served as control.

Following fixation the tissues from the above four groups were divided into two parts. Those intended for morphological studies were embedded in paraffin, cut and stained with haematoxylin-eosin. The others intended for the cytochemical demonstration of acid phosphatase (E C 3.1.3.–) were prepared by a modified Gomori procedure as described in detail elsewhere (3).

Control sections which were incubated in a similar Gomori medium containing 0.01 M NaF showed no enzyme activity.

The osmolality of the various solutions was measured by freezing-point depression using a Knauer Halb Micro osmometer with a 400 mOsm NaCl solution as a reference.

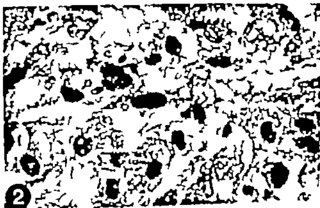


Fig. 2. Decidual cells exposed to hypertonic saline injected extra-amniotically 15 minutes before vacuum aspiration. Several cells show pyknotic nuclei and vacuolated cytoplasm. H E ×485

RESULTS

Material from control patients

Decidual cells from patients not treated with hypertonic saline showed well preserved morphology and a generally distinct granular distribution of the enzyme reaction product (Figs. 1 and 4).

Material from saline treated patients

In the material obtained by hysterotomy the parietal decidua showed alterations of the same type and approximately the same magnitude whether the hypertonic saline was injected *intra-amniotically* or extra-amniotically.

Morphology. Pyknotic nuclei and cytoplasmic vacuoles appeared in decidual cells in part of the decidua 15 minutes following injection of the hypertonic saline (compare Figs. 1 and 2). The extent of these changes varied with the interval between the injection of the hypertonic saline and the evacuation of the uterine contents. In almost all decidual cells obtained after saline abortion the nuclei were disintegrating and the cytoplasm showed extensive vacuolization and dissolution (Fig. 3).

Acta Obstet Gynec Scand 53 275–277 1974

CASE REPORTS

FETAL PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA REPORT OF A CASE DOCUMENTED BY TRANS-VAGINAL ELECTROCARDIOGRAM DURING FETAL DISTRESS IN LABOUR

Nicola G Carretti, Paolo A. Galli and Pierandrea Pellegrino

From the Department of Obstetrics and Gynaecology (Head. Prof A. Centaro) and Department of Pediatrics (Head Prof E. Sartori) University of Padua, Italy

Abstract. A case is reported of fetal paroxysmal supraventricular tachycardia detected during fetal distress. The trans-vaginal technique was used for the electrocardiographic recording. Fetal distress was demonstrated by measuring fetal blood pH taken from the scalp and umbilical cord. During 2-hour recording, before caesarean section was performed, paroxysmal supraventricular tachycardia of 300 b/min and atrial fibrillation were observed The ECG of the infant was completely normal 8 hours after birth

Many authors (1 2, 3 4) studying fetal electrocardiogram (ECG) with abdominal electrodes noticed an electric activity similar to fetal ECG waves, with a frequency between 300 and 800 beats per minute (b/min) and abnormal rhythms

Such electrical activities have been the subject of controversial discussions and uncertainty Some authors (5) maintain there are no sure proofs to demonstrate the authenticity of the cardiac origin of these activities.

The study of fetal ECG with the trans-vaginal technique and application of electrodes to the fetal scalp (6, 7) allows clear vision of only the fetal complex, excluding superimpositions and distortions Moreover the trans-vaginal technique gives the possibility to monitor fetal heart activity during the entire labour period and eventually to compare differing findings in the same fetus

The report illustrates a case of tachyarrhythmia detected by the trans-vaginal technique

CASE REPORT

On April 20th 1971 healthy 4-year-old housewife entered the clinic in early labour during the 38th week of her second pregnancy

Amnioscopy showed green amniotic fluid. The fetal heart beat, as determined by auscultation was arrhythmic and bradycardic (80–120 b/min) Amniotomy and infusion of 5 I.U of oxytocin (Syntocinon) were promptly carried out. The clinical symptoms prompted a first determination of fetal acid–base equilibrium: the pH was 7 23. Labour continued and the electrode was applied to the fetal scalp at cervical dilatation of 4 centimetres. Recording of the fetal ECG was continued for about 2 hours. The alterations in fetal ECG indicated need for a second microblood test, the pH was 7 14 A caesarean section was promptly performed and the pH of the blood from the umbilical cord was 7 10. The new born infant of 3 150 g, female Apgar 6/1 was resuscitated (Apgar 8/5) and kept in thermostatic cradle for 48 hours. An ECG was done 8 hours after birth. The technique used for the fetal ECG is well known (6 7).

ECG FINDINGS

The fetal ECG showed a succession of 3 fundamental changes of different duration which will be reported according to the order of their appearance.

At the beginning of the recording (Fig. 1 A) the frequency remained within normal limits, varying between 120–160 b/min The QRS has a high voltage it is broad and slurred, this finding was not observed subsequently

Fig. 1 B is characterized by the presence of many small waves of very high frequency (600 b/min) appearing in the intervals between the QRS complexes which have a normal morphology The ventricular rate averages 70 b/min but is very irregular with variable R–R interval (a–b in Fig. 1 B) thus showing the features of an atrial fibrillation with very slow ventricular rate.

Subsequently the R–R interval suddenly shortens becomes regular and paroxysmal supraventric

din $F_{2\alpha}$ is liberated into amniotic fluid following extra amniotic injection of hypertonic saline (13) and (*b*) both extra-amniotic (6 17 18) and intra amniotic (4 16) administration of this compound induces abortion These observations together with those mentioned above make it probable that one of the factors involved in the mode of action of hypertonic saline is the release of prostaglandins from damaged decidual tissue

ACKNOWLEDGEMENTS

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REFERENCES

1 Bengtsson L Ph Therapeutic abortion by means of intra-uterine injections Techniques effects risk and mechanism of effect. Med Gynecol Sociol 3 No 1 1968

2. Bengtsson L. Ph & Ryde E Legal abort genom intra-uterina injektioner (2): En analys av lunda-materialet 1960–1966. Läkartidn 64 5049 1967

3 Brunk U & Gustavii B Lability of human decidual cells In vitro effects of autolysis and osmotic stress. *Amer J Obstet Gynecol 115 811 1973*

4 Bygdeman M Toppozada M & Wiqvist N Induction of midtrimester abortion by intra-amniotic administration of prostaglandin $F_{2\alpha}$ Acta Physiol Scand 82 415 1971

5 de Duve C & Wattiaux R Functions of lysosomes Ann Rev Physiol 28. 435 1966.

6. Embrey M P & Hillier K Therapeutic abortion by intrauterine instillation of prostaglandins. Br Med J 1 588 1971

7 Ericsson J L. E & Brunk, U Lysosomal membranes as related to diseases process. *In* Pathologic aspects of cell membranes (ed B F Trump and A U Arstila). Academic Press, New York 1973 (in press)

8 Gustavii, B Studies on accidental intravascular injection in extra-amniotic saline induced abortion and a method for reducing this risk. J Reprod Med 8, 70, 1972.

9 — Labour A delayed menstruation? Lancet 1 1149 1972.

10 — Intra-amniotic and extra-amniotic injection of sodium chloride solution Amniotic fluid salinity and abortifacient effect. Amer J Obstet Gynecol (in press)

11 Gustavii, B & Brunk U Decidual-cell necrosis after injection of hypertonic saline for therapeutic abortion Lancet 2 826, 1971

12. — A histological study of the effect on the placenta of intra-amniotically and extra-amniotically injected hypertonic saline in therapeutic abortion. Acta Obstet Gynecol Scand 51 1 1 1972.

13 Gustavii B & Green K Release of prostaglandin $F_{2\alpha}$ following injection of hypertonic saline for therapeutic abortion A preliminary study Amer J *Obstet Gynecol 114 1099 1972.*

14 Jansson P O Nilsson, L. Spetz, S & Swarting, P Legal abort genom intra-uterina injektioner (4). Intra- eller extra-amnial instillation of hyperton NaCl? Läkartidn 64 5146 1967

15 Karim S. M M & Devlin J Prostaglandin content of amniotic fluid during pregnancy and labour J Obstet Gynaec Br Comm 74 230 1967

16. Karim S M M & Sharma, S D Second trimester abortion with single intra-amniotic injection of prostaglandins E_2 or $F_{2\alpha}$ Lancet 2 47 1971

17 Wiqvist, N Béguin, F Bygdeman, M Fernström, I & Toppozada, M Induction of abortion by extra-amniotic prostaglandin administration. Prostaglandins 1 37 1972

18 Wiqvist, N & Bygdeman, M Therapeutic abortion by local administration of prostaglandin Lancet 2 716, 1970

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CASE REPORTS

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Many authors (1 2 3 4) studying fetal electrocardiogram (ECG) with abdominal electrodes noticed an electric activity similar to fetal ECG waves with a frequency between 300 and 600 beat per minute (b/min) and abnormal rhythms.

Such electrical activities have been the subject of controversial discussions and uncertainty Some authors (5) maintain there are no sure proofs to demonstrate the authenticity of the cardiac origin of these activities.

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The report illustrates case of tachyarrhythmias detected by the trans-vaginal technique.

CASE REPORT

On April 20th 1971 healthy 24-year-old housewife entered the clinic in early labour during the 33th week of her second pregnancy

Amnioscopy showed green amniotic fluid. The fetal heart beat, as determined by auscultation, was arrhythmic and bradycardic (80–120 b/min). Amniotomy and infusion of 5 I U of oxytocin (Syntocinon) were promptly carried out. The clinical symptoms prompted a first determination of fetal acid–base equilibrium, the pH was 7.23 Labour continued and the electrode was applied to the fetal scalp at cervical dilatation of 4 centimetres. Recording of the fetal ECG was continued for about 2 hours. The alterations in fetal ECG indicated need for second microblood test. the pH was 7 14. A caesarean section was promptly performed and the pH of the blood from the umbilical cord was 7 10. The newborn infant of 3 150 g, female, Apgar 6/1 was resuscitated (Apgar 8/5) and kept in thermostatic cradle for 48 hours. An ECG was done 8 hours after birth. The technique used for the fetal ECG is well known (6, 7).

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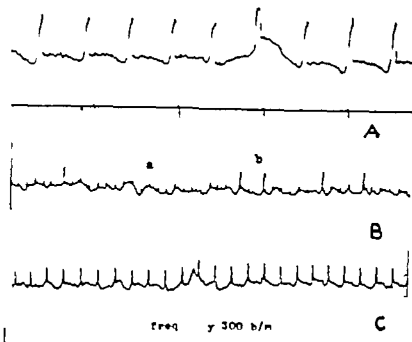


Fig 1 (A) Trans-vaginal ECG during labour Frequency 150 b/min high voltage broad and slurred QRS complexes and uncertain P waves. (B) Atrial fibrillation (atrial rate 600 b/min ventricular rate 70 b/min). (C) Paroxysmal supraventricular tachycardia (300 b/min)

ular tachycardia (PST) can clearly be seen (Fig 1 C) with a frequency of 300 b/min lasting about 30 sec and followed by the slow atrial fibrillation At this point the recording was stopped

Eight hours after birth the ECG was normal (Fig 2) with sinus rhythm and a frequency of 130 b/min There were no signs of heart disease and the child was healthy at 6 months of age

COMMENT

Even if the ECG alterations observed have 3 main different features the problem is to see if they all *depend upon only one common cause* namely the fetal distress

The first ECG feature was seen before the first alteration of the pH value At this moment it was

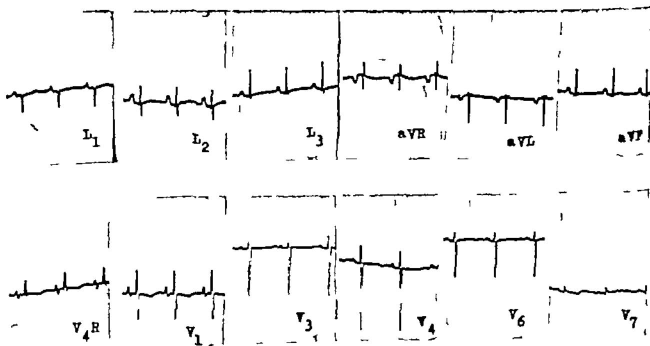


Fig 2 ECG 8 hours after birth

not possible to make a diagnosis of sinus rhythm because there are no clearly visible P waves related to the QRS complexes. In any case the frequency of QRS complexes is regular and remains within those limits compatible with normal heart function although their morphology appears broad and slurred.

Later at the time of the first pH alteration the change of the ECG tracing was noted. These two features might be considered as typical of atrial fibrillation, but it is not completely possible to exclude that the small waves are a technical artefact. Against this hypothesis there is the consideration that these features never appeared in the preceding and succeeding tracings.

Fig. 1 C where a regular succession of QRS complexes with a frequency of 300 b/min is observed, represents, without doubt a typical example of PST. This is, as far as we know the first case documented by direct ECG of the occurrence of this kind of arrhythmia in the human fetus with fetal distress during labour.

An important point to consider is the chronological correspondence between the progressive decrease of the fetal metabolic values and the fetal ECG alterations (8). On the contrary at the beginning of recording when the pH was 7.23 the ventricular frequency was regular and after birth the infant had a normal ECG and clinic status.

Many authors (9, 10, 11) reported that fetal deaths may occur in tachycardia, according to this the PST can be considered as an event of short duration which can come before fetal death as the cardiac function is strictly dependent on the ventricular rate. In our case in fact it is possible to state that the PST has a sure meaning of functional cardiac distress and it might be directly dependent on the acid base equilibrium alterations, with pH values down to 7.10 or might be considered consequence of some local myocardial modifications.

In conclusion the most important finding of our report is the observation of a PST documented by direct fetal ECG. The association of this kind of arrhythmia and of the other observed alterations with the fetal distress biochemically documented does not permit us to state which of the two pathological events caused the other.

REFERENCES

1. Reymorts, J., Hildebrand, W., Montauban van Swijndregt, L. Traces de tachyarythmie cardiaque foetale in utero. Realité ou artéfact? Bull Féd Soc Gynécol Obstet Franc *13* 572, 1967.
2. Smith, C. N. Experimental electrocardiography of the foetus. Lancet *1* 1124 1953.
3. Hildebrand, W. F. Some abnormal foetal electrocardiograms. Medical Electronics (ed. C. N. Smith Iliffe) p. 43 London, 1960.
4. Southern, E. M. Electrocardiography and phonocardiography of the foetal heart. J Obstet Gynecol Brit Emp *61* 231 1954.
5. Sureau, C., Trocellier, R., Chavinie, J., Cannon, M. Le diagnostic électrocardiographique de tachy-arythmie foetale. Gynécol Obstet (Paris) *63* 149 1964.
6. Carretti, N. Su alcuni aspetti tecnici di E.C.G. fetale in travaglio di parto. Atti Soc Med Chir Padova *18* 15 1968.
7. Hon, E. H. Instrumentation of the foetal heart rate and feotal electrocardiography. II. A signal electrode. Am J Obstet Gynecol *86* 772, 1963.
8. Wood, C., Ferguson, R., Leeton, J., Newman, W. & Walker, A. Fetal heart rate and acid-base status in assessment of fetal hypoxia. Am J Obstet Gynecol *98* 6, 1967.
9. Hon, E. H., Lee, S. T. The foetal electrocardiogram. the electrocardiogram of the dying foetus. Am J Obstet Gynecol *87* 804 1963.
10. Blumenthal, S., Jacobs, J. C., Steer, C. M. & Williamson, S. W. Congenital atrial flutter: report of a case documented by intra-uterine electrocardiogram. Pediatrics *42* 659 1968.
11. Carretti, N. L'elettrocardiogramma fetale per via trans-vaginale: descrizione di due casi di alterazione del ritmo in travaglio di parto. Att Ost Ginecol *18* 64 1968.

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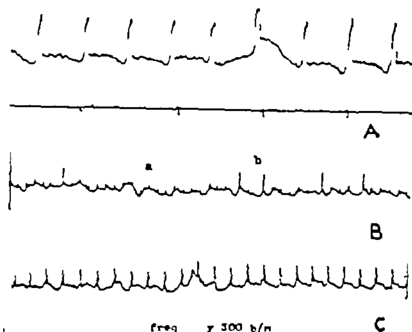


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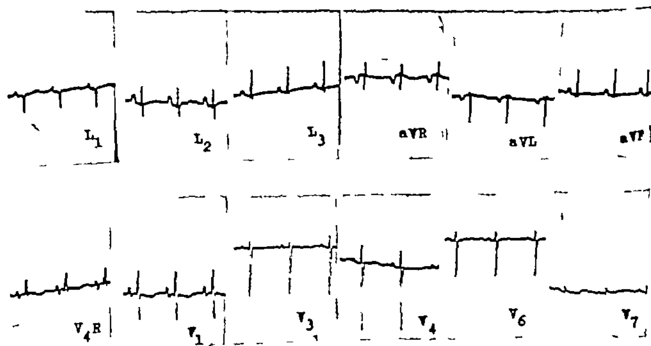


Fig 2 ECG 8 hours after birth

Acta Obstet Gynec Scand 53 279–282, 1974

COMPLICATIONS FOLLOWING THE INTRA AMNIOTIC ADMINISTRATION OF PROSTAGLANDIN $F_{2\alpha}$ FOR THERAPEUTIC ABORTION

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Abstract. A case of intra-amniotic prostaglandin $F_{2\alpha}$ injection of mid-trimester abortion is reported, where tachycardia, hypothermia and hypotension developed as complications. When induction was completed by an intravenous oxytocin infusion, rupture of the cervix occurred in connection with the abortion.

Since the publication of the first reports on the use of prostaglandin (PG) $F_{2\alpha}$ (15) and E_2 (16) as abortifacients, several clinical trials have been carried out (for review see 14). Various routes of administration have been used for therapeutic abortion. Intra-amniotic injection seems preferable to extraamniotic (13) and intravenous (24) administration, if the efficacy of induction and the frequency of side-effects are considered.

The most common side-effect is gastrointestinal distress including nausea, vomiting, and diarrhoea as well as flushing. In one case of attempted intra-amniotic induction $PGF_{2\alpha}$, which was accidentally given into the extra-amniotic space caused vomiting, dyspnoea, cyanosis and nasal congestion (5). Fever has been reported quite frequently as a side-effect (12, 22). Bronchospasm, a rare but potentially serious complication, has also been described (9). PG s may affect blood coagulation (2), and many of the PG s are potent inhibitors of platelet aggregation (19). PGE_2 has also been reported to induce a sickle-cell crisis (23). Nevertheless, no fatal complications reported to be caused by PG s are known to the authors.

The present paper is a case report of unusual complications following the intra-amniotic administration of $PGF_{2\alpha}$ for therapeutic abortion

CASE REPORT

The patient was a 24-year-old, unmarried, healthy waitress, whose height was 166 cm and weight 56 kg, and who had a normal menstrual history. She had had no previous deliveries or abortions. The patient was admitted into our clinic at the 17th week of pregnancy as calculated from the last menstrual period, for abortion because of a social indication. Her pregnancy had been normal, and examination showed the cervix to be normal and the size of the uterus to correspond to 17 week pregnancy.

An intra-amniotic induction was performed as follows. After evacuation of the bladder an amniocentesis was made halfway between the symphysis and the fundus uteri under local anaesthesia (10 ml of 1 % Xylocaine). A thin polyethylene cannula was threaded into the amniotic cavity through the needle. The needle was removed and clear amniotic fluid without blood was obtained through the cannula. 40 mg of $PGF_{2\alpha}$ was injected into the amniotic cavity. 1 1/2 hours after the induction the patient felt some weakness and paraesthesiae in her trunk and limbs. After 20 minutes these symptoms disappeared spontaneously for a moment, but 2 hours after the $PGF_{2\alpha}$ injection the patient began to sweat and turned pale. Uterine contractions became very strong, and the patient vomited, while at the same time her blood pressure fell from 115/70 mmHg to 90/60 mmHg (Fig. 1). The heart rate fell to 46/min. Axillary temperature went down to 34.6°C and rectal temperature to 35.1°C. The patient was given 40 mg of pethidine intramuscularly. The situation returned to normal in one hour. The heart rate went up to 60/min, the blood pressure to 120/70–80 mmHg, and the axillary temperature to 36.5°C, while the uterine contractions became weaker. After 24 hours, an intravenous infusion of oxytocin was started (30 I U of oxytocin in 1 000 ml of saline at a rate of 2.5 I.U./hour). 35 hours after the induction and 11 hours after the beginning of oxytocin infusion, the patient was delivered. The fetus was a foot presentation and the fetal feet and body were delivered through the dilated external

ANNOUNCEMENTS

German Society for Endocrinology Competitions for 1975

Schoeller–Junkmann Award (DM 15 000 –) Donator Schering AG Berlin Applicants must reside in Europe and not be older than 40 years. Subjects. All fields of endocrinology except diabetes mellitus)

Marius Tausk Career Development Award (DM 15 000 –) Donator Organon G m b H Munich Applicants must reside in Europe and not be older than 33 years Subjects Clinical and clinical-experimental endocrinology (except diabetes mellitus)

Applicants are invited to submit two copies of previously unpublished papers in either German or English together with a short curriculum vitae and a description of the development of their scientific career to the President of the German Society for Endocrinology for 1974/75 Prof Dr med. vet H Karg Süddt Versuchs- und Forschungsanstalt für Milchwirtschaft Weihenstephan Technische Hochschule München Institut fur Physiologie 8050 Freising not later than October 15 1974

After receipt of the manuscript has been acknowledged by the German Society for Endocrinology the author is at liberty to have his paper published by a periodical.

Detailed information concerning the awards may be obtained from the President of the Society The awards will be presented at the 21st Symposium of the German Society for Endocrinology 1975

The 17th Interim Congress of Obstetricians & Gynaecologists

will take place in Johannesburg, South Africa from 30th September to 3rd October 1974 and coincides with the Golden Jubilee of the Medical School of the University of the Witwandersrand.

Further information can be obtained from the Secretary Doctor A. Rubin The South African Society of Obstetricians and Gynaecologists Dept of Obstetrics & Gynaecology New Medical School, Esselen Street, Johannesburg R.S.A 2001

The VIII World Congress on Fertility and Sterility

will take place in Buenos Aires November 3-9 1974.

Official themes.

1. Mechanism of action of hormones (Moderator Dr J Rosner)
2. Immunological aspects of reproduction (Moderator Dr I Halbrecht).
3. Male fertility (Moderator Dr R E. Mancini).
4. Recent progress (Moderator Dr P C Steptoe).
5. Fertilization and early embryonic developments including detrimentals genetic and exo-genetic factors (Moderator Dr A Ingelman-Sundberg)
6. New developments in fertility control (Moderator Dr S J Segal)
7. New developments in the neuroendocrinology of reproduction (Moderator Dr L. Martini).
8. New technical developments in therapeutic and diagnostic procedures (Moderator Dr K Semm)
9. Neuroendocrine components of sex behaviour (Moderator Dr C Beyer).

Main lectures

1. Impact of basic research on the management of reproduction *(Prof K. J Ryan)*
2. Mode of action of hormones (Prof E E Baulieu).
3. Immunological aspects of reproduction (Prof. K. Bratanov).
4. Current research on male reproductive function (Prof T Mann).
5. Physiology and physiopatology of the tube (Prof E. M Coutinho)

Postgraduate courses.

1. Functional exploration of sterility
2. Endocrine aspects of sterility
3. Surgery and fertility
4. Physiological aspects of fertility
5. Genetics and reproduction.
6. Male sterility
7. Malformations of the female genital tract and fertility
8. Ethical, religious and legal aspects in the study of fertility
9. Reproduction of research.

Contributions of the official themes.
Workshops
Video-tape-films-special tapes

Secretariat Cenl S A Av R. Sáenz Peña 1110 2° piso Buenos Aires, Argentina

side-effects of $PGF_{2\alpha}$ in the present case, impurities in the solutions used must also be considered. The fact that we have subsequently given $PGF_{2\alpha}$ from the same batch by the same route without similar side-effects appearing seems to eliminate the possibility of impurities existing in the preparation

The duration of the reaction after an intra-amniotic injection of PG may be longer than that following intravenous administration, because PG is absorbed slowly from the amniotic cavity and its metabolism may be retarded (4) The possibility of a cardiovascular reaction during PG induction should be taken into account and caution may be needed in cases of cardiovascular disease.

In our case there was a further complication a rupture of the cervix, when the PG induction was completed by oxytocin infusion. Injuries of the cervix following induced mid-trimester abortion including intra-amniotic injection of $PGF_{2\alpha}$ have also been reported earlier and a careful digital and speculum examination after induced abortion is therefore recommended (3). Larger doses have lately been used for the intra-amniotic administration of PG in order to improve the success rate Even single doses of 40–100 mg of $PGF_{2\alpha}$ have been used (5) in which case repeated injections are mostly unnecessary But large doses of PG s may induce very intense contractions and thereby increase the associated risks of uterine and cervical injuries

REFERENCES

1 Änggård E & Bergström, S Biological effects of an unsaturated trihydroxyacid ($PGF_{2\alpha}$) from normal swine lung Acta Physiol Scand 58 1 1963.

2 Badraoui, M H H Bonnar J Hillier K & Embrey M P Coagulation changes during termination of pregnancy by prostaglandins and by vacuum aspiration Br Med J 1 19 1973

3 Bradley-Watson, P J Beard R J & Craft I L Injuries of the cervix after induced midtrimester abortion J Obstet Gynaecol Br Commw 80 284 1973

4 Caldwell B V Anderson, G G Hobbins, J C & Speroff L F prostaglandin levels in women receiving $PGF_{2\alpha}$ *I* Prostaglandins in fertility control Reports from a conference on analytical procedures metabolism and clinical evaluation January 18–20 1972 in Stockholm WHO research and training centre on human reproduction (ed S. Bergström, K Green and B Samuelsson), p 182 Karolinska Institutet Stockholm, 1972

5 Craft, I Intra-amniotic prostaglandin E_2 and $F_{2\alpha}$ for induction of abortion dose-response study J Obstet Gynaecol Br Commw 80 46, 1973.

6. DuCharme D W., Weeks, J R. & Montgomery R. G Studies on the mechanism of the hypertensive effect of prostaglandin $F_{2\alpha}$ J Pharmacol Exp Ther 160 1 1968.

7 Eckt, M. Gauer O H Kaptein, F & Lange L. Effects of prostaglandin E_1 and $F_{2\alpha}$ on cutaneous arteries and veins in vivo in the isolated rabbit ear *I* Advances in the Biosciences 9 (ed. S. Bergström and S. Bernhard). International Conference on Prostaglandins, Vienna, September 25–28, 1972, p. 353 Pergamon Press Vieweg, Braunschweig, 1973

8. Ferreira, S. H. & Vane J R. Prostaglandins their disappearance from and release into the circulation. Nature 216 868, 1967

9 Fishburne, J I Jr Brenner W E., Braaksma, J T & Hendricks, C H Bronchospasm complicating intravenous prostaglandin $F_{2\alpha}$ for therapeutic abortion Obstet Gynecol 39 892, 1972

10. Gillett, P G Kinch R. A H Wolfe L. S. & Pace Asciak, C. Induction of abortion by intra-amniotic $PGF_{2\alpha}$ comparison of dose schedules. *In* Advances in the Biosciences 9 (ed. S. Bergström and S. Bernhard) International Conference on Prostaglandins, Vienna, *September 25–28, 1972, p. 545* Pergamon Press, Vieweg, Braunschweig, 1973

11 Hedqvist P Prostaglandin mediated control of sympathetic neuroeffector transmission. *I* Advances in the Biosciences 9 (ed. S. Bergström and S. Bernhard). International Conference on Prostaglandins Vienna, September 25–28, 1972 p. 461 Pergamon Press Vieweg, Braunschweig, 1973

12. Hendricks, C H. Brenner W E. Ekbladh L. Brotanek, V & Fishburne, J I Jr Efficacy and tolerance of intravenous prostaglandins $F_{2\alpha}$ and E_2. Amer J Obstet Gynaecol 111 564 1971

13 Ilärvinen, P A Pentinnen, S & Ylöstalo P Induction of abortion by intra- and extra-amniotic prostaglandin $F_{2\alpha}$ administration. Prostaglandins 3 491 1973

14 Karim, S M M Prostaglandins and human reproduction Physiological and clinical uses of prostaglandins in relation to human reproduction *In* The Prostaglandins, Progress in Research (ed S M. M Karim) p 71 MTP Oxford and Lancaster 1972

15 Karim, S M M & Filshie G. M. Therapeutic abortion using prostaglandin $F_{2\alpha}$. Lancet I 157 1970

16. — Use of prostaglandin E_2 for therapeutic abortion Br Med J 3 198 1970

17 Karim, S M M Sharma, S D Filshie G M Salmon, J A & Ganesan P A Termination of pregnancy with prostaglandin analogs *I* Advances in the Biosciences 9 (ed S Bergström and S Bernhard) International Conference on Prostaglandins Vienna, September 25–28, 1972, p 811 Pergamon Press Vieweg, Braunschweig, 1973

18. Karim, S M. M & Somers K Cardiovascular and renal actions of prostaglandins *I* The Prostaglandins, Progress in Research (ed S M. M Karim) p 165 MTP Oxford and Lancaster 1972.

19 Kloeze J Influence of prostaglandins on platelet adhesiveness and platelet aggregation *In* Prostaglan-

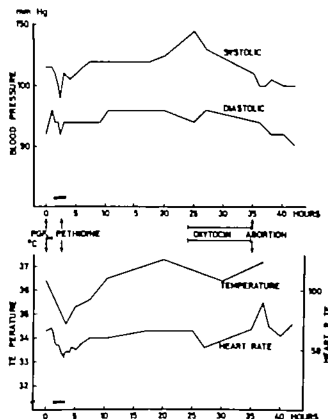


Fig 1 Systolic and diastolic blood pressures axillary temperature and heart rate after intra-amniotic administration of 40 mg of $PGF_{2\alpha}$ to the patient ☐=weakness and numbness ⩘= very strong uterine contractions A pethidine dose of 40 mg was given intramuscularly

cervical os. The fetal head however was expelled through a rupture of the cervix. The rupture was in the upper dorsal part of the cervical canal and opened into the posterior fornix of the vagina. The neck of the foetus was cut, and the fetus was removed in two parts Evacuation and curettage of the uterus and suture of the rupture followed. It was observed that there was intact cervical tissue about 1/2 cm broad between the rupture and the external os. After evacuation the patient recovered normally

DISCUSSION

The cardiovascular effects of $PGF_{2\alpha}$ (for review see 18) differ markedly in different animal species. In man however slow intravenous infusion of up to 20 mg of PGF_2 /hour has no effect on the cardiovascular system Up to 50 mg of $PGF_{2\alpha}$ has been given rectally or per vaginam without any significant cardiovascular effect. This lack of influence is probably due to the rapid uptake and/or metabolism of $PGF_{2\alpha}$ by the lungs and the liver as well as its rapid diffusion from the blood (8) Our patient who received $PGF_{2\alpha}$ intra-amnioti cally developed marked bradycardia and hypotension Both of these reactions are opposite to those which $PGF_{2\alpha}$ is assumed to cause theoretically We suggest that they are not direct actions of $PGF_{2\alpha}$ but might be due to vagal stimulation. There is an earlier report on vasovagal reactions not described precisely in 3 out of 20 patients after the intra-amniotic administration of $PGF_{2\alpha}$ (10) In animal experiments PG s (PGE_2 and PGA_1) have been found to lower the blood pressure probably through central vagal stimulation and it was possible to prevent this by atropine or vagotomy (20). $PGF_{2\alpha}$ causes a fall of arterial blood pressure and marked bradycardia in the cat Bradycardia is probably of reflex origin and is therefore prevented by atropine and vagotomy (1)

On the other hand PGE_1 and PGE_2, and to a lesser extent also $PGF_{2\alpha}$ are able to interfere with the release of noradrenaline from sympathetic nerve endings after stimulation of the sympathetic nerves This inhibition can be seen in various tissues, e g isolated mammalian heart (11) It can be postulated that due to the vigorous contractions of the uterus, pain caused a sympathetic-adrenal stimulation but the release of the transmitter was prevented by $PGF_{2\alpha}$ and the cardiovascular effects could not be restored

Hypothermia which was noted in our patient has not been reported earlier in connection with the use of PG s in obstetrics Intravenous $PGF_{2\alpha}$ causes venoconstriction (6) and contracts the veins and arteries in the rabbit ear if administered in concentrations normally used in man (7). Karim et al (17) who tested the efficacy of different 15(S)- and 15(R)-15 methyl derivatives of PGE_2 methyl esters described feeling cold and shivering as a fairly common transient side-effect, but unfortunately the authors did not report the body temperatures of the patients No reaction of this kind was however seen after corresponding analogues of $PGF_{2\alpha}$ Roberts et al (21) observing the effects of intravenous infusion of prostaglandin $F_{2\alpha}$ noted cyanosis in the hand and forearm where the infusion was being given and a fall of the finger tip temperature The authors suggest an intense contraction of the smooth muscle of the venous wall as the cause of reduced blood flow They also suggest that intravenous administration of $PGF_{2\alpha}$ should be contraindicated in patients with a history of peripheral vascular disturbances

As a possible explanation for the unexpected

side-effects of $PGF_{2\alpha}$. In the present case impurities in the solutions used must also be considered. The fact that we have subsequently given $PGF_{2\alpha}$ from the same batch by the same route without similar side-effects appearing seems to eliminate the possibility of impurities existing in the preparation.

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In our case there was a further complication, a rupture of the cervix, when the PG induction was completed by oxytocin infusion. Injuries of the cervix following induced mid-trimester abortion including intra-amniotic injection of $PGF_{2}\alpha$ have also been reported earlier and a careful digital and speculum examination after induced abortion is therefore recommended (3). Larger doses have lately been used for the intra-amniotic administration of PG in order to improve the success rate. E.g. single doses of 40–100 mg of $PGF_{2\alpha}$ have been used (5), in which case repeated injections are mostly unnecessary. But large doses of PG's may induce very intense contractions and thereby increase the associated risks of uterine and cervical injuries.

REFERENCES

1. Änggård, E. & Bergström, S. Biological effects of an unsaturated trihydroxyacid (PGF2α) from normal swine lung. Acta Physiol Scand 58: 1. 1963.
2. Bodmon, M. H. H., Bonnar, J., Hilbert, K. & Embrey, M. P. Coagulation changes during termination of pregnancy by prostaglandins and by vacuum aspiration. Br Med J 1: 19. 1973.
3. Bradley-Watson, P. J., Beard, R. J. & Craft, I. L. Injuries of the cervix after induced midtrimester abortion. J Obstet Gynaecol Br Commw 80: 284. 1973.
4. Caldwell, B. V., Anderson, G. G., Hobbins, J. C. & Speroff, L. F prostaglandin level in women receiving PGF2α. I. Prostaglandins in fertility control. Reports from conference on analytical procedures, metabolism and clinical evaluation. January 18–20, 1972 in Stockholm. WHO research and training centre on human reproduction (ed. S. Bergström, K. Green and B. Samuelsson), p. 182. Karolinska Institutet, Stockholm, 1972.
5. Craft, I. Intra-amniotic prostaglandin E2 and F2α for induction of abortion: dose-response study. J Obstet Gynaecol Br Commw 80: 46. 1973.
6. DuCharme, D. W., Weeks, J. R. & Montgomery, R. G. Studies on the mechanism of the hypertensive effect of prostaglandin F2α. J Pharmacol Exp Ther 160: 1. 1968.
7. Echt, M., Gauer, O. H., Kapteina, F. & Lange, L. Effects of prostaglandin E2 and F2α on cutaneous arteries and veins in vivo in the isolated rabbit ear. I. Advances in the Biosciences 9 (ed. S. Bergström and S. Bernhard). International Conference on Prostaglandins, Vienna, September 25–28, 1972, p. 353. Pergamon Press, Vieweg, Braunschweig, 1973.
8. Ferreira, S. H. & Vane, J. R. Prostaglandins: their disappearance from and release into the circulation. Nature 216: 868. 1967.
9. Fishburne, J. I., J., Brenner, W. E., Braaksma, J. T. & Hendricks, C. H. Bronchospasm complicating intravenous prostaglandin F2α for therapeutic abortion. Obstet Gynecol 39: 892, 1972.
10. Gillett, P. G., Kinch, R. A. H., Wolf, L. S. & Pace-Asciak, C. Induction of abortion by intra-amniotic PGF2α: comparison of dose schedules. In Advances in the Biosciences 9 (ed. S. Bergstrom and S. Bernhard). International Conference on Prostaglandins, Vienna, September 25–28, 1972, p. 345. Pergamon Press, Vieweg, Braunschweig, 1973.
11. Hedqvist, P. Prostaglandin mediated control of sympathetic neuroeffector transmission. I. Advances in the Biosciences 9 (ed. S. Bergström and S. Bernhard). International Conference on Prostaglandins, Vienna, September 25–28, 1972, p. 461. Pergamon Press, Vieweg, Braunschweig, 1973.
12. Hendricks, C. H., Brenner, W. E., Ekbladh, L., Brotanek, V. & Fishburne, J. I., Jr. Efficacy and tolerance of intravenous prostaglandins F2α and E2. Amer J Obstet Gynaecol 111: 564, 1971.
13. Järvinen, P. A., Pennanen, S. & Ylöstalo, P. Induction of abortion by intra- and extra-amniotic prostaglandin F2α administration. Prostaglandins 3: 491. 1973.
14. Karim, S. M. M. Prostaglandins and human reproduction. Physiological and clinical uses of prostaglandins in relation to human reproduction. I. The Prostaglandins, Progress in Research (ed. S. M. M. Karim), p. 71. MTP, Oxford and Lancaster. 1972.
15. Karim, S. M. M. & Filshie, G. M. Therapeutic abortion using prostaglandin F2α. Lancet I: 157. 1970.
16. — Use of prostaglandin E2 for therapeutic abortion. Br Med J 3: 198. 1970.
17. Karim, S. M. M., Sharma, S. D., Filshie, G. M., Salmon, J. A. & Ganesan, P. A. Termination of pregnancy with prostaglandin analogs. I. Advances in the Biosciences 9 (ed. S. Bergström and S. Bernhard). International Conference on Prostaglandins, Vienna, September 25–28, 1972, p. 811. Pergamon Press, Vieweg, Braunschweig, 1973.
18. Karim, S. M. M. & Somers, K. Cardiovascular and renal actions of prostaglandins. I. The Prostaglandins. Progress in Research (ed. S. M. M. Karim), p. 165. MTP, Oxford and Lancaster. 1972.
19. Kloeze, J. Influence of prostaglandins on platelet adhesiveness and platelet aggregation. I. Prostaglan-

dins Proceedings of the Second Nobel Symposium Stockholm, June 1966 (ed S. Bergström and B Samuelsson) p 241 Almqvist & Wiksell Stockholm 1967
20 Leach B E Muirhead E. E, Armstrong, F B & Germain G S Vasopressor action of renomedullary prostaglandins (PGE_2 and PGA_2) nin the spontaneously hypertensive rat (SH rat). Evidence for a central action mediated by the vagus Advances in the Biosciences 9 (ed S Bergström and S. Bernhard) International Conference on *Prostaglandins, Vienna,* September 25–28, 1972, p. 369 Pergamon Press Vieweg Braunschweig 1973
21 Roberts G Mottram R. F Parry H & Bloom A Cyanosis due to intravenous prostaglandin $F_{2\alpha}$ Lancet 2 425 1972.
22. Seppälä, M Kajanoja, P Widholm O & Vara, P Prostaglandin-oxytocin abortion a clinical trial on *intra-amniotic prostaglandin* $F_{2\alpha}$ *in combination* with intravenous oxytocin. Prostaglandins 2 311 1972.
23 Willis A. L. Johnson, M., Rabinowitz, I & Wolf, P L. Prostaglandin E_1 may induce sickle-cell crisis. N Engl J Med 286 783 1972.
24 Wiqvist, N Bygdeman M & Toppozada, M Induction of abortion by the intravenous administration of prostaglandin $F_{2\alpha}$ Acta Obstet Gynecol Scand 50 381 1971

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LETTER TO THE EDITOR

DIAGNOSING CORYNEBACTERIUM VAGINALE (HAEMOPHILUS VAGINALIS) BY MEANS OF WET MOUNTS AND DIRECT STAINED SMEARS

In their recent study in this Journal (4) Doctors Åkerlund and Mårdh concluded that *Corynebacterium vaginale* (*Haemophilus vaginalis*) could not be diagnosed with certainty by wet mounts or direct stained smears. This challenging conclusion was presented as a statement without resorting to pertinent numerical data which strangely are absent from their whole study

The material of this study on *Corynebacterium vaginal* (*Haemophilus vaginalis*) is not extensive containing only 22 (refined material 16) isolations of the organism from the cervical canal of the study subjects The authors choice in using samples from the cervical canal only for isolation of a vaginal micro-organism is extraordinary It also appears that in search for "clue cells" Gram-stained cervical secretion was used The futility of searching for specific vaginal epithelial cells (2) from cervical secretions is manifest.

It may be recalled that all the studies which the authors give as references for the diagnostic value of wet mounts (1 2) direct Gram-stained smears (1 2), and Papanicolaou smears (3) contain material from the vaginal content

It is obvious that the study of Doctors Åkerlund and Mårdh does not qualify as a reference study on the diagnostic value of wet mounts direct Gram-stained smears and Papanicolaou smears concerning *Corynebacterium vaginale* (*Haemophilus vaginalis*)

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REFERENCES

1. Brewer J. L., Halpern, B. & Thomas, G. *Haemophilus vaginalis* vaginitis. Am J Obstet Gynecol 74 834 1957
2. Gardner H. L. & Dukes, C. D. *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "non-specific" vaginitis. Am J Obstet Gynecol 69 962, 1955
3. Lewis J F & O'Brien S Diagnosis of *Haemophilus vaginalis* by Papanicolaou smears. Am J Clin Pathol 51 412, 1969
4. Åkerlund, M. & Mårdh, P.-A., Isolation and identification of *Corynebacterium vaginale* (*Haemophilus vaginalis*) in women with infections of the lower genital tract. Acta Obstet Gynecol Scand 53 85 1974

Acta Obstet Gynec Scand 53 285–286 1974

Reply to Letter

The Possibility of Diagnosing Lower Genital Tract Infections with Coryn bacterium Vaginale by means of Wet Mounts and St ined Smears

In a study presented in the last issue of this Journal (4) in which the method of Dunkelberg et al (1) wa used for the identification of C aginale this bacterium was isolated from 31% of 70 patient with symptoms and signs of lower genital tract infection (LGTI) but from none of 28 healthy controls *Inter alia* the study gave an appraisal of the value of wet mounts Gram and Papanicolaou stained smears in the diagnosis of C aginale

The letter by Dr Leppäluoto does not qualify as a critical e aluation of our study since hi criticism is based on complete misinterpretation of ou data. He finds our conclusion that a LGTI by C aginale cannot be diagnosed with certainty by et mount or stained smears as challenging We feel that Dr Leppaluoto contention that one is able to identify as to species gram-negative rod in aginal secretion by the mere study of wet mount or stained smears is untenable particularly in iew of the fact that he does not present any data of his own in support

Dr Leppaluoto feels that our study of C vaginale is not extensive Our communication contains thorough and intensi e study of 98 patient including clinical findings isolation rates findings in wet mount and stained smears as well a bacteriological characteristics of C vaginale its growth on arious media, gas chromatography analysis of metabolites of C aginale and antibiotic susceptibility of the bacterium In our opinion it is better to present a limited number of thoroughly investigated cases than a great number of badly defined ones Dr Leppäluoto reference to refined cases we feel is not relevant to the matter under discussion Dr Leppaluoto states that our study is presented without reserting to pertinent numerical data It is not surprising that Dr Leppäluoto, who apparently read our report superficially has not observed that it contains more than 70 numerical data in the section concerning the patients only

We find it strange that Dr Leppäluoto regards C vaginale as a vaginal micro-organism Perhaps the Christian name of the organism might have given Dr Leppaluoto his impression that C vaginale is strictly confined to the vagina We hope that a reading of our study will convince him that it is not In this connection it would be worthwhile to cite a recent study of C vaginale n males (2). For bacteriological examination of patients with LGTI we consider cervix to be a more appropriate source for sampling than vagina.

We are unable to see how Dr Leppäluoto got the impression that we used Gram stained cervical secretion for the study of clue cells As clearly described in our study (4) page 86 wet mount were made from material from the posterior ag inal fornix We agree with Dr Leppaluoto that it is futile to search for vaginal epithelial cells in cervical secretion. Naturally for clue cells we looked t the smears from the portio and the posterior vaginal fornix The attached figure shows a clue cell in wet mount from the posterior vaginal fornix of one of our patients who did not harbour C vaginale as evidenced by repeated cultures Other cells from this patient were covered even more intensely with bacteria. A further evaluation of a cytological diagnosis of C vaginale has also been gi en elsewhere (3)

It is clear therefore from our data, that there was no strict correlation between the occurrence of clue cells nd C vaginale The use of more strict criteria for the bacteriological diagnosis of C aginale (1) s used by us may very well explain the difference between our results and those referred by Dr Leppäluoto

Even *a priori* t is hard to accept the argument that C aginale possesses the unique capability of

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THE RELATIONSHIP BETWEEN UTERINE VOLUME, PLASMA PROGESTERONE AND INTRAUTERINE PRESSURE

A Preliminary Report

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From the Departments of Obstetrics and Gynecology (Head: Professor L. Rauramo) and Clinical Chemistry (Head: B. Laurent, M.D.), University of Turku, Turku, Finland

Abstract. In a group of 8 midtrimester pregnant patients, the uterine volume (V) had been increased by the intra-amniotic instillation of 150 ml Macrodex and the changes in plasma progesterone (P) and intrauterine pressure (IUP) had been measured. In 2 of these 8 women the study was discontinued because of the early rupture of the fetal membranes. In 4 patients the P levels decreased slightly and IUP increased, culminating in abortion. In 2 patients the P levels increased distinctly, the IUP remained unchanged and pregnancy continued undisturbed. These findings support Csapo's conclusion that the ratio V/P controls IUP and thus the fate of pregnancy.

The concept (1) that the ratio uterine volume/progesterone (V/P) is a major controlling factor of the intrauterine pressure (IUP) and thus the fate of pregnancy has gained considerable support during recent years (for *references* see 2). Through this concept it can be predicted that increasing V promotes the evolution of IUP (through stretch effect), unless this activating action of V is balanced by a proportional increase in P. Therefore, Csapo considered that the rising P levels during gestation represent a placental compensatory effort towards the promotion of pregnancy (demanded by the continued increase in V) and the terminal failure in this compensatory effort is a preparatory step towards the initiation of labor (1, 3, 4, 6).

Thus, it can also be predicted that in midtrimester patients a therapeutic increase in V should trigger the evolution of IUP and abortion, unless time is allowed for a placental compensatory response resulting in increased progesterone production. In order to test the validity of this premise we increased V in 8 nulliparous patients (by the intra-amniotic installation of a high molecular weight dextran solution), measured the subsequent changes in P and IUP and observed clinical progress in abortion. An earlier study already showed (7) that sustained increase in V triggers the evolution of IUP and abortion in certain cases, but without P assays it offered no explanation for those cases in which abortion failed to occur.

THE STUDY: PATIENTS AND METHODS

A group of 8 nulliparous patients, 20±0.5 years old (mean±S.E.) and 15±0.5 weeks pregnant, were studied. Normal plasma P levels (36 ng/ml) indicated normal pregnancy. During a period of 15–30 minutes 350 ml Macrodex (Astra AB, Sweden, containing 1 mg/ml tetracycline) was injected into the amniotic sac by transabdominal puncture. As a prophylactic measure 800 000 IU penicillin, with 1 g streptomycin, was given intramuscularly. The intrauterine pressure (IUP) has been recorded sequentially by the extraovular microballoon technique (5), and the active pressure (AP), resting pressure (RP), oxytocin response (OR) calculated from the tracings. The changes in uterine volume were described earlier (7). Plasma P was measured (8) before volume increase, and subsequently at daily intervals.

RESULTS

Of the 8 study patients 4 aborted (Figs. 1 and 2) following a decrease in plasma P and an increase in AP and OR. The remaining 4 patients did not abort. In 2 cases, an increase (58 and 64%) in

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Vaginal epithelial cell with small coccoid bacteria on its surface ("clue cell") from a woman with negative cultures for Corynebacterium vaginale Wet mount of specimen from the posterior vaginal fornix (phase contrast ×800)

forming "clue cells" i e attach to the surface of vaginal epithelial cells which will distinguish it from all other species of bacteria possibly present in the lower genital tract of women with LGTI

In our series "clue cells" were found in about one third of the patients from whom C vaginale was not isolated

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REFERENCES

1 Dunkelberg, W E Jr Skaggs, R & Kellogg, D S Jr Method for isolation and identification of Corynebacterium vaginale (Haemophilus vaginalis). Appl Microbiol 19 47 1970
2. Mårdh P-A. & Colleen S Search for evidence of urogenital tract infections in patients with symptoms of non-acute prostatitis Scand J Nephrol Urol, in press.
3 Mårdh P A Stormby N & Åkerlund, M The value of Papanicolaou stained vaginal smears in the diagnosis of infections with Corynebacterium vaginale (Haemophilus vaginalis) Acta Cytol (Philad) in press.
4 Åkerlund, M & Mårdh P A., Isolation and identification of Corynebacterium vaginale (Haemophilus vaginalis) in women with infections of the lower genital tract, Acta Obstet Gynecol Scand 53 85 19

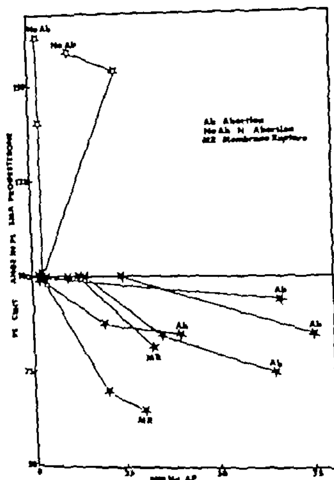


Fig 2 Correlation between plasma progesterone and intrauterine pressure after volume increase in 8 midtrimester patients.

(*b*) the osmotic increase in V (after Macrodex treatment) is slow enough to permit the time dependent increase in progesteronegenesis,

( ) the V-triggered increase in IUP and clinical progress in abortion does not suppress placental endocrine function

Further experiments, in which V is increased at different rates and amounts, are needed to verify this tentative interpretation of the results reported bove

These proposed studies can clarify whether or not the increased placental synthesis of progesterone during pregnancy is promoted by gradually increasing V (2). Increasing V can terminate pregnancy through stretch-induced prostaglandin synthesis (6), unless this effect is balanced by increased P levels (2)

ACKNOWLEDGEMENTS

The authors are indebted to Prof. A. Csapo for the benefit of discussions, to Mrs Marjatta Sutonen for assistance in IUP recording and data analysis, and to Miss Eva Saxen for assistance in P analysis.

REFERENCES

1 Csapo, A. L. *I* Progesterone and the Defence Mechanism of Pregnancy (ed. G. E. W. Wolstenholme and M. P. Cameron) Ciba Foundation Study Group No. 9 J & A. Churchill, London, 1961.

– Csapo, A. I *I* Progesterone Its Regulatory Effect on the Myometrium (ed. G. E. W. Wolstenholme and J. Knight), Ciba Foundation Study Group No. 34. J & A Churchill London, 1969

3. Csapo, A., Knobil, E., Pulkkinen, M., Van der Molen, W. J. Sommerville, I. F. & Wiest, W. Amer J Obstet Gynecol 105. 1132, 1969

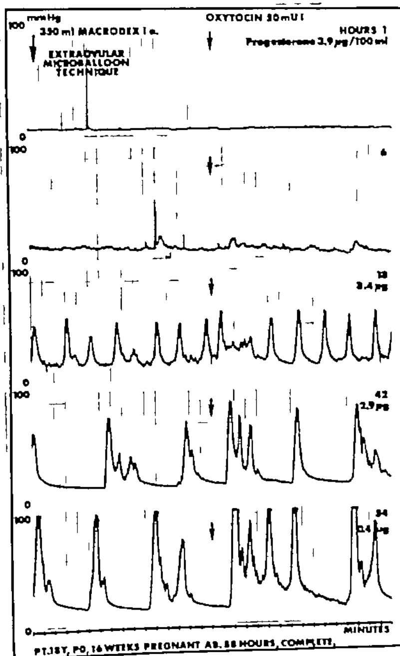


Fig 1 Original recording in a successful case of induction by macrodex installation in midtrimester pregnancy Plasma progesterone in the upper right hand corner of the corresponding panel (micrograms per 100 ml of plasma).

plasma P was measured and no increase in AP or OR (Figs. 3 and 2) In the remaining 2 pa tients the membranes ruptured early (? hrs after volume increase) and the cases were removed from the study In the 4 patients who aborted (after a decrease in plasma P) the RP slightly increased, while in those 2 patients whose plasma P increased (by about 60%) and who did not abort there was no change in RP (Fig. 4)

DISCUSSION

In 4 (out of 8) patients an increase in uterine volume (V) induced increased RP AP and OR a decrease in plasma P and abortion. In contrast in 2 patients, whose plasma P increased after an increase in V the RP AP and OR did not increase and pregnancy continued undisturbed. The remaining patients were removed from the study after early membrane rupture

These preliminary findings supports the concept (1 2) that increasing V may induce a compensatory synthesis of progesterone in the placenta, provided that

(*a*) the osmotic action of the injected hypertonic solution (Macrodex) does not suppress placental function to a degree equal to that of the stimulatory effect of increasing V

4 Csapo, A. I. Sauvage, J. & Wiest, W. Amer J Obstet Gynecol 108 950, 1970.
5 Csapo, A. I. Obstet Gynecol Survey 25 515 1970
6 Csapo, A. I. J Reprod Med 9 400, 1972.
7 Pulkkinen, M. O. Liukko, P. Piiroinen, O. & Rauramo, L. Acta Obstet Gynecol Scand 52, 9 1973
8 Wilman, K. J Clin Lab Invest Suppl 110 129 1969

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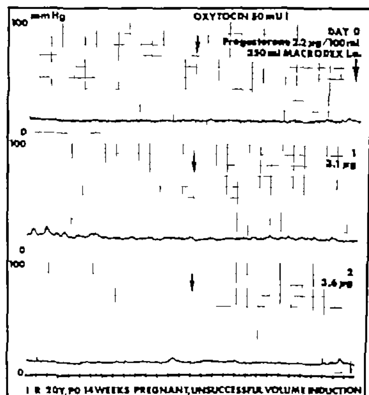


Fig 3 Original recording in a successful case of induction by macrodex installation in midtrimester pregnancy Plasma progesterone in the upper right hand corner of the corresponding panel (micrograms per 100 ml of plasma).

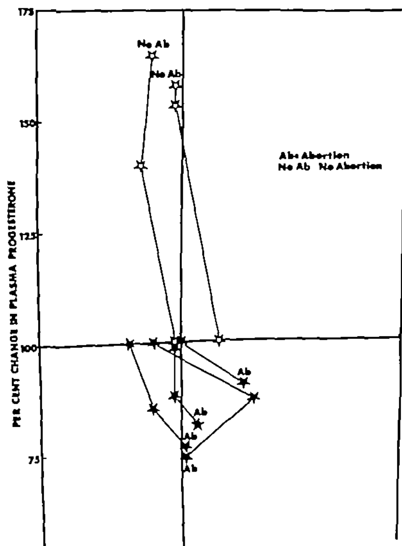


Fig 4 Correlation between plasma prog... and resting p... of ...

Acta Obstet Gynec Scand 53 293–302, 1974

CERVICAL CONSISTENCY IN WOMEN OF FERTILE AGE MEASURED WITH A NEW MECHANICAL INSTRUMENT

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Abstract. A new instrument designed for the measurement of the cervical consistency has been used in an investigation of 100 non-pregnant and 49 pregnant women. The instrument consists of a mechanism for control of the force used, enabling the examiner to exert the desired pressure on the uterine cervix, and another mechanism for recording the tissue consistency. Both mechanisms were calibrated before each measurement. During measurement the instrument was held horizontally and pressed lightly against the anterior lip of the cervix. The cervical consistency is characterized by the angle α between the walls of the impressed surface recorded on the indicator mechanism of the instrument. The highest values of α are found in the softest tissues. The results showed highly significant inter-individual differences ($F = 7.355$, P [illegible]) between 30 of the non-pregnant patients who had from 3 to 6 measurements performed in different menstrual cycles (intraclass correlation $= 0.612 \pm 0.083$). Variation about the individual mean is only $1/F = 13.6\%$. In the non-pregnant cervix a rise in the mean measured values was noted during the first phase of the menstrual cycle. The mean values for non-pregnant women using combined oral contraceptives were slightly higher. In normal pregnancies the measurements showed significantly different values in three different time intervals. The highest values were found near term. Within a few weeks after parturition the values fall and approached the normal values for non-pregnant women.

The consistency of the uterine cervix can be estimated by palpation. This is a subjective method, but the impression of great individual differences is obvious to everyone who is performing this examination. From a theoretical point of view the consistency pattern might differ as far as age groups, menstrual cycle, parity, hormone medication and gestational age in pregnancy are concerned. Objective measurements of the cervical consistency have not been made before, and the reason for this may be the lack of suitable instruments for the assessment of tissue consistency.

An instrument designed for measurement of the consistency of the uterine cervix (1) has been used in this investigation. The elastic property of the cervix is here called the fibro-elasticity of the cervical tissue.

Anatomy and Physiology

The vaginal part of the uterine cervix projects into the vagina through the upper end of the anterior wall. In nulligravidae it is usually 2.5 to 3 cm in length and only slightly less in diameter and it is noted to increase in size as a result of parity or infection (15). The normal position of the cervix is directed downward and backward, and the degree of angulation is variable. The external os is more or less circular in the nulligravida, but in the parous female it is usually observed as a transverse slit which frequently is fissured. The anterior lip is shorter and thicker than the posterior due to the line of vaginal attachment, and with the cervix in its usual position the anterior lip projects lower than the posterior.

Stratified squamous epithelium covers the uterine cervix. The epithelium is made up of several layers conventionally described as: basal, parabasal, intermediate and superficial. The thickness of the squamous epithelium is dependent upon several factors such as glucogen content, degree of oestrogenic stimulation and desquamation of surface cells (7). It is of interest in the present investigation that a 0.03 micron thick basement membrane has been demonstrated with the electron microscope (10).

The columnar epithelium of the cervical canal varies in thickness from 1.2 to 3.5 mm in the

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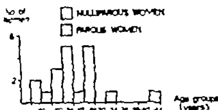


Fig. 2. Age distribution of 19 women using combined oral contraceptives.

Table III. *Parity distribution in the group of 49 pregnant women*

No. of children	No. of women	Calculated frequency	Statistical evaluation
0	23	20.80	$M = 0.8571$
1	15	17.82	$s^2 = 1.0417$
2	7	7.64	$\chi^2 = 0.7173$
3	3	2.18	d f = 2
4	1	0.47	$P_{.50} < \chi^2 < P_{.70}$

in Fig. 3. The measurements were done monthly from the first time the woman came for a routine examination in pregnancy until her 32nd week. In the eighth month two examinations were performed, and in the last month of pregnancy the examinations were undertaken weekly. Another reason for the unequal number of measurements performed in the different periods of pregnancy was that some women applied for an examination late in pregnancy or moved from the district before the series of examinations was completed.

The cervical changes are supposed to be more marked in the multigravida than in the nulliparous woman (10), and it is therefore of interest to know the nullipara/para ratio when the mean values of the cervical consistency are calculated. The nullipara/para ratio among the pregnant women was 23/26. These two groups have been compared (see Results). The 49 pregnant women together had had 42 children, and the parity distribution is shown in Table III. The mean number *M* of children per woman is 0.86 ± 0.15. This number *M* has been used in calculating the expected frequency in the Poisson distribution (see Table III). There was very good agreement between the observed and the calculated frequencies (χ^2 0.717, d.f. 2). The proportion of primigravidae (23/49 = 46.9%) is also in agreement with the calculated (e^{-M} = 42.4%) and there is no significant difference between s^2 and M (s^2/M = 1.016 (Table III)).

In two pregnancies abnormal conditions were detected (twins, toxaemia).

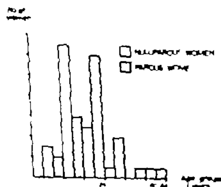


Fig. 3. Age distribution of the 49 pregnant women in the material.

Fourteen nulliparous and 21 parous women were also examined after parturition, in order to analyse the involution process expressed by the cervical consistency.

METHODS

To a certain extent, the human portio vaginalis uteri may be compared to a visco-elastic material in technical terminology having both plastic and elastic properties, which can be characterized by *softness* and *fibro-elasticity* respectively. In the cervix the fibro-elasticity seems to dominate, since the test results obtained with the uterine cervix closely resemble the results for fibro-elastic materials as described in detail elsewhere (3).

The anterior lip of the cervix was used for the measurements (Fig. 4). Vaginal discharge was wiped away before the measurement so that the indicator mechanism of the instrument should not be exposed to the vaginal fluid.

1. The equipment

In Fig. 5 the instrument and its different parts is shown. It has suitable dimensions for *vaginal examinations and* is constructed for horizontal use. The instrument does not cause any discomfort or injury to the patient and can be sterilized.

The instrument has one mechanism which enables the examiner to exert the same pressure from the instrument to the cervical tissue each time (the axial force *control mechanism*), and another mechanism for registration of tissue consistency (indicator mechanism).

The instrument is made of a steel bar with the axial

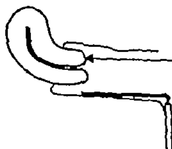


Fig. 4. Arrow shows position of instrument and place of measurement on the anterior lip of uterine cervix.

Table I *Distribution of measurements between the non-pregnant and the pregnant women*

Group	Parity	No. of women	No of measurements
Non-pregnant with normal menstrual cycles	Nulliparous	15	17
	Parous	66	162
Non-pregnant on combined oral contraceptive	Nulliparous	6	11
	Parous	13	28
Pregnant	Nulliparous	23	107
	Parous	26	153
Post-partum	(Parous)	35	45

Table II *Measurements performed on 30 women who were monitored from 3 to 6 normal menstrual cycles*

Menstrual cycle	No. of women	No. of measurements
I	30	34
II	30	32
III	30	30
IV	14	15
V	8	8
VI	1	2

non pregnant state. Under the hormonal stimulus of gestation the mucosa doubles in thickness and secretes a thick, opaque and viscous mucus (12, 17)

Danforth (5) found the cervix of the human uterus to be composed predominantly of fibrous connective tissue with an average of 15% of smooth muscle He found minute and insignificant amounts of elastic fibres, which were sparsely scattered in a haphazard manner throughout the substance of the cervix. Berwind (2) studied the collagen fibres of the cervix in pregnant and non-pregnant women The thickness of the fibrils seemed to increase with advancing age The consistency of the non-pregnant cervix appeared to depend first of all on the consistency and the degree of polymerization of the ground substance and not on the fibrils. Depolymerization of the ground substance causes both softening of the cervical tissue and better displacement of the fibrils. The water concentration in the cervix increases significantly during pregnancy reaching a maximum immediately prior to labour and the accumulation of water amounts to an increase of 7 % (3) Retention of water and electrolytes and loosening of the acid mucopolysaccharides of the binding ground substance are well known effects of oestrogens (13 16).

The hypertrophic and hyperplastic changes of the uterine and cervical blood and lymph vessels during pregnancy are pronounced (4 6). At the end of pregnancy the cervix is like a sponge.

The innervation of the cervix originates in three plexuses of the pelvic autonomic system. Krantz (14) has demonstrated free nerve endings entering a papilla of the stratified squamous epithelium of the uterine cervix.

The present investigation was undertaken to measure the fibro-elasticity of the uterine cervix in regular menstrual periods, in women using a combined oral contraceptive and in normal pregnancies.

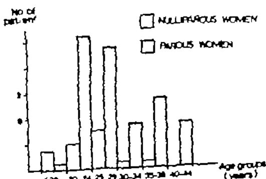


Fig 1 Age distribution of 15 nulliparous and 66 parous women with normal menstrual cycles.

MATERIAL

I this investigation 100 non-pregnant and 49 pregnant women with no visible cervical pathology were examined. Table I shows the distribution of the women in the series, and also the number of measurements performed in the different groups.

The age distribution of the non pregnant women is shown in Figs. 1 and 2. The menstrual cycles in 81 women (Fig. 1) lasted 23 to 30 days, called "normal cycles" Regular ovulations were demonstrated by a rise in the body temperature in one woman who had a continuous temperature chart. Three other women were measured in the menstrual cycles from 14 to 103 days before they became pregnant Measurements were executed in 3 to 6 cycles in 30 of the 81 women with normal cycles (Table II). Nineteen women (Fig. 2) were using combined oral contraceptives containing 0.5 mg norgestrel and 0 05 mg ethinyloestradiol (Eugynon ®).

The age distribution of the pregnant women is shown

to what was found when the instrument was tested on other materials (1).

During the measurement, the instrument was in contact with the cervix for about 2 seconds. Three consecutive measurements were performed, but only the first value has been used in this investigation since vaginal fluid has disturbed the other two readings by changing the frictional force between the indicator wings.

2. Statistical methods and notations

Variance analysis was performed on the recorded values from 30 non-pregnant women who had measurements performed with the instrument in 3 or more menstrual cycles (Table III). It is of interest to know if there is difference between the measured values from the different women. The intraclass correlation, the coefficient of variation about the individual mean have therefore been calculated. The t-test was used for comparison of the measured values of the non-pregnant nulliparous and parous women, and furthermore between the values of the non-pregnant nulliparous women with normal menstrual cycles and those women using the contraceptive pill.

For the pregnant women the mean number of measurements and the harmonic mean have been calculated, and the values recorded in early, middle and late pregnancy have been compared. The measurements which were performed after the parturition have been correlated to the values of the non-pregnant women.

The statistical symbols and equations used in this publication are listed below (8, 11, 19)

- N Number of patients
- k Number of measurements or number of children in subsample
- x Measured value
- $M, \bar{x}, \bar{k}$ Arithmetic mean of variates
- Md Median (50 % of f_o)
- f Expected number or calculated number
- f Observed number
- d.f. Degrees of freedom
- $S(x-\bar{x})^2$ Sum of squared deviations
- s^2 Variance $= S(x-\bar{x})^2/\text{d.f.}$
- s Standard deviation $= \sqrt{s^2}$
- C.V. Coefficient of variation in per cent $= 100\,(s/M)$
- t Student's t-test $= (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{s^2 \left(\frac{1}{k} + \frac{1}{k_2}\right)}$ or tabulated value of t
- P Percentile point of frequency distribution
- Skewness $= 3\,(M - Md)/s$
- $s_{\bar{x}}$ Standard error of the mean $= s/\sqrt{k}$
- s^2 Pooled variance $= \dfrac{S(x_1-\bar{x}_1)^2 + S(x_2-\bar{x}_2)^2}{k + k_2 - 2}$
- M_h Harmonic mean $= N/S\frac{1}{k}$
- $t \cdot s_{\bar{x}}$ 95 % confidence interval
- s_b^2 Variance between patients
- s_w^2 Variance within patients
- T Ratio $= s_b^2/s_w^2 = (F-1)/k$
- F Fisher's test $= kT+1 = [1+(k-1)r]/(1-r)$

Intraclass correlation $= s_b^2/(s_b^2 + s_w^2) = T/(T+1)$

Coefficient of intraclass correlation based on k measurements

$$= (F-1)/F = T/\left(T+\frac{1}{k}\right) = kT/(kT+1)$$

$$= \frac{s^2}{s^2 + s_w^2/k}$$

- e^{-m} The expected number in class 0 of the Poisson distribution
- K Proportion of variance due to variation between measurements within the same patient $= 1/F = 1/(kT+1) = 1 - r_k$
- k Number of measurements required by stated value of K or F and given T $= \left(\frac{1}{K}-1\right)\frac{1}{T} = (F-1)\frac{1}{T}$
- $\bar{k}$ Average number of k in subsamples used in analyses of variance $= \left[Sk - \frac{Sk^2}{Sk}\right]\frac{1}{N-1}$
- r_T Weight index for subsample mean based on k measurements $= (T+1)/\left(T+\frac{1}{k}\right) = 1/r_k$
- r_k Variance between subsample mean based on k measurements relative to variance based on one measurement per patient $\left(T+\frac{1}{k}\right)/(T+1) = 1/r_T$

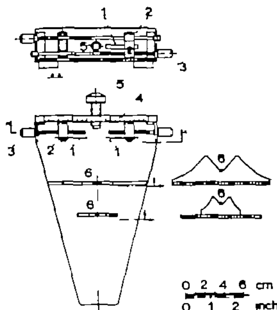


Fig. 7. The instrument for calibration of indicator mechanism. 1. Lamella springs of beryllium copper foil. 2. Supports for lamella springs. 3. Screw for control of distance between lamella springs. 4. Scale for control of distance between lamella springs. 5. Screw for control of distance behind lamella springs. 6. Supports for alignment of instrument to be calibrated.

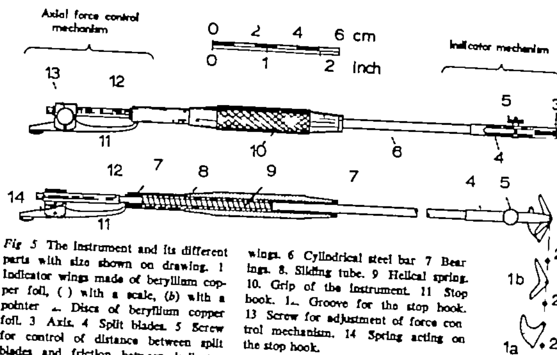


Fig 5 The instrument and its different parts with size shown on drawing. 1 Indicator wings made of beryllium copper foil, ( ) with a scale, (*b*) with a pointer 2 Discs of beryllium copper foil. 3 Axis. 4 Split blades. 5 Screw for control of distance between split blades and friction between indicator wings. 6 Cylindrical steel bar 7 Bearings. 8. Sliding tube. 9 Helical spring. 10. Grip of the instrument. 11 Stop hook. 1_ Groove for the stop hook. 13 Screw for adjustment of force control mechanism. 14 Spring acting on the stop hook.

force control mechanism at one end and the indicator mechanism at the other end. The tension of a helical spring in the force control mechanism is controlled by a calibrated stop mechanism, and to this is mounted a stop hook which signals by a click" when the desired force is applied during measurement. The indicator end of the instrument is formed like an Indian ink pen, and the "nib opening is controlled by a screw Two indicator wings rotate on a thin spindle which connects the ends of the two split blades. The wings are formed as sectors of a circle, and on one of the wings is a scale calibrated in scale units from 1 to 10, making a total of 75 angle degrees. When the instrument is held by the grip and the indicator end is pressed lightly against the convexity of the anterior lip of the cervix, the sliding tube moves toward the indicator end. During this procedure, the indicator wings slide over one another The angle between them depends on the consistency of the cervix in such a way that the angle decreases with increasing softness of the tissue. When the desired force has been applied, the measurement is completed and the instrument withdrawn. The measured value can be read on the indicator scale under a magnifying glass.

Before each measurement the instrument was calibrated. Fig. 6 shows the instrument and the equipment used for calibration. The force control mechanism was calibrated by pressing the indicator end of the instrument against a balace when the instrument was held by the grip in a horizontal position. The indicator mechanism was calibrated in a special construted instrument (see Fig. 7). Each of the indicator wings was simultaneously pressed against two lamella springs, one lamella spring for each indicator wing During this procedure the indicator wings slide over one another because of the spring forces from the lamellae, and the friction between the indicator wings was adjusted so that the value of 8 scale units was read on the indicator scale when the calibration was completed.

The instrument was adjusted to "0 g force in the experiments presented in this publication. In a test series where 10 g force was used, the friction in the indicator mechanism disturbed the measurements. This corresponds

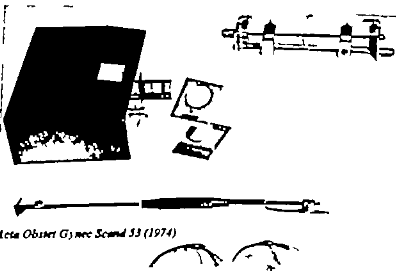


Fig 6 The equipment used in the investigation. Upper left balance for calibration of force control mechanism Upper right: instrument for calibration of indicator mechanism. Centre: magnifying glass. The instrument with indicator ad force control mechanism is shown below in the picture.

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Measured value

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Md Median (50% of f_o)

f Expected number or calculated number

f Observed number

d.f. Degrees of freedom

$S(x-\bar{x})^2$ Sum of squared deviations

s^2 Variance $= S(x-\bar{x})^2/\mathrm{d.f.}$

Standard deviation $= \sqrt{s^2}$

C.V. Coefficient of variation in per cent $= 100\ (s/M)$

Student' t-test $= (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{s^2\ \left(\frac{1}{k_1} + \frac{1}{k_2}\right)}$ or tabulated value of t

P Percentile point of frequency distribution

Skewness 3 (M Md)/s

$s_{\bar{x}}$ Standard error of the mean $s/\sqrt{k}$

s^2 Pooled variance $= \frac{S(\bar{x}_1 - x_1)^2\ \ S(\bar{x}_2 - x_2)^2}{k_1 + k_2 - 2}$

M Harmonic mean $= N/\Sigma\frac{1}{k}$

$M_{-t, +t}$ 95% confidence interval

s^2 Variance between patients

Variance within patients

T Ratio s_b^2/s^2 $(F-1)/k$

F Fisher's test $= kT + 1 = [1 + (k-1)\ r_I]/1 - r_I$

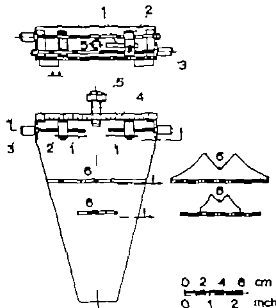


Fig. 7. The instrument for calibration of indicator mechanism. 1. Lamella springs of beryllium copper foil. 2. Supports for lamella springs. 3. Screw for control of distance between lamella springs. 4. Scale for control of distance between lamella springs. 5. Screw for control of distance behind lamella springs. 6. Supports for alignment of instrument to be calibrated.

Intraclass correlation $= s_b^2/(s^2 + s_b^2) = T/T+1$

Coefficient of intraclass correlation based on k measurements

$=(F\ \ 1)/F = T/\left(T\ \ \frac{1}{k}\right) = kT/(kT+1)$

$= \frac{s_b^2}{s_b^2 + s_w^2/k}$

e^{-m} The expected number in class 0 of the Poisson distribution

K Proportion of variance due to variation between measurements within the same patient $= 1/F = 1/(kT+1) = 1 -$

k Number of measurements required by stated value of K or F and given T

$= \left(\frac{1}{K} - 1\right)\ \frac{1}{T} = (F\ \ 1)\ \frac{1}{T}$

k Average number of k in subsamples used in analysis of variance $= \left[Sk\ \ \frac{Sk}{Sk}\right]\ \frac{1}{N\ \ 1}$

r_T Weight index for subsample mean based on k measurements $= (T+1)/\left(T + \frac{1}{k}\right) = 1/s_T$

s_T^2 Variance between subsample mean based on k measurements relative to variance based on one measurement per patient

$= \left(T + \frac{1}{k}\right)/(T+1) = 1/r$

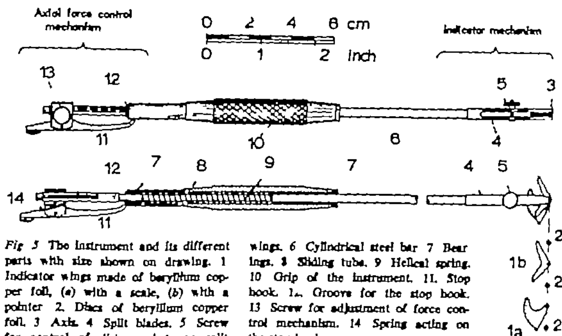


Fig 5 The instrument and its different parts with size shown on drawing. 1 Indicator wings made of beryllium copper foil, (*a*) with a scale, (*b*) with a pointer 2. Discs of beryllium copper foil. 3 Axis. 4 Split blades. 5 Screw for control of distance between split blades and friction between indicator wings. 6 Cylindrical steel bar 7 Bearings. 8 Sliding tube. 9 Helical spring. 10 Grip of the instrument. 11. Stop hook. 12. Groove for the stop hook. 13 Screw for adjustment of force control mechanism. 14 Spring acting on the stop hook.

force control mechanism at one end and the indicator mechanism at the other end. The tension of a helical spring in the force control mechanism is controlled by a calibrated stop mechanism, and to this is mounted a stop hook which signals by a click" when the desired force is applied during measurement. The indicator end of the instrument is formed like an Indian ink pen, and the "nib opening is controlled by a screw Two indicator wings rotate on a thin spindle which connects the ends of the two split blades. The wings are formed as sectors of a circle, and on one of the wings is a scale calibrated in "scale units" from 1 to 10, making a total of 75 angle degrees. When the instrument is held by the grip and the indicator end is pressed lightly against the convexity of the anterior lip of the cervix, the sliding tube moves toward the indicator end. During this procedure, the indicator wings slide over one another The angle between them depends on th consistency of the cervix in such a way that the angle decreases with increasing softness of the tissue. When the desired force has been pplied, the measurement is completed and the instrument withdrawn. The measured value can be read on the indicator scale under a magnifying glass.

Before each measurement the instrument was calibrated. Fig. 6 shows the instrument and the equipment used for calibration. The force control mechanism was calibrated by pressing the indicator end of the instrument against a balace when the instrument was held by the grip in a horizontal position. The indicator mechanism was calibrated in a special constructed instrument (see Fig. 7) Each of the indicator wings was simultaneously pressed against two lamella springs, one lamella spring for each indicator wing. During this procedure the indicator wings slide over one another because of the spring forces from the lamellae, and the friction between the indicator wings was adjusted so that the alue of 8 scale units was read on the indicator scale when the calibration was completed

The instrument was adjusted to 20 g force in the experiments presented in this publication. In a test series where 10 g force was used, the friction in the indicator mechanism disturbed the measurements This corresponds

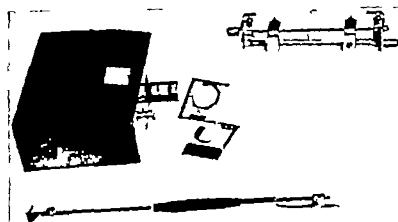


Fig 6 The equipment used in the investigation. Upper left. balance for calibration of force control mechanism. Upper right: instrument for calibration of indicator mechanism. Centre magnifying glass. The instrument with indicator and force control mechanism is shown below in the picture.

to what was found when the instrument was tested on other materials (1).

During the measurement, the instrument was in contact with the cervix for about 2 seconds. Three consecutive measurements were performed, but only the first value has been used in this investigation since vaginal fluid has disturbed the other two readings by changing the frictional force between the indicator wings.

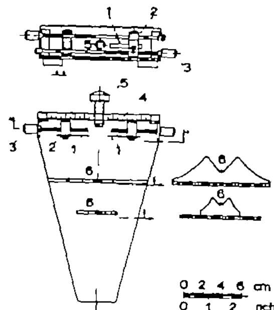


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2. *Statistical methods and notations*

Variance analyses was performed on the recorded values from 30 non-pregnant women who had measurements performed with the instrument in 3 or more menstrual cycles (Table III). It is of interest to know if there is difference between the measured values from the different women. The intraclass correlation, the coefficient of variation about the individual mean have therefore been calculated. The t test was used for comparison of the measured values of the non-pregnant nulliparous and parous women, and furthermore between the values of the non-pregnant nulliparous women with normal menstrual cycles and those women using the contraceptive pill.

For the pregnant women the mean number of measurements and the harmonic mean have been calculated, and the values recorded in early, middle and late pregnancy have been compared. The measurements which were performed after the parturition have been correlated to the values of the non-pregnant women.

The statistical symbols and equations used in this publication are listed below (8, 11, 19).

N Number of patients

k_i Number of measurements or number of children in subsample

x Measured value

M $\bar{x}$, $\bar{k}$ Arithmetic mean of variates

Md Median (50% of f_o)

f Expected number or calculated number

f Observed number

df Degrees of freedom

$S(x-\bar{x})^2$ Sum of squared deviations

s^2 Variance $= S(x-\bar{x})^2/df$

s Standard deviation $\sqrt{s^2}$

CV Coefficient of variation in per cent $= 100\ (s/M)$

t Student t-test $(\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\bar{s}^2 \left(\frac{1}{k_1} + \frac{1}{k_2}\right)}$ or tabulated value of t

P Percentile point of frequency distribution

Skewness $3\ (M - Md)/s$

$s_{\bar{x}}$ Standard error of the mean $s/\sqrt{k}$

$\bar{s}^2$ Pooled variance $\dfrac{S(x_1 - \bar{x}_1)^2 + S(x_2 - \bar{x}_2)^2}{k_1 + k_2 - 2}$

M Harmonic mean $N/S\frac{1}{k}$

$M \pm t_{.05}\,s_{\bar{x}}$ 95% confidence interval

s_b^2 Variance between patients

s_w^2 Variance within patients

T Ratio $s_b^2/s^2 = (F-1)/k$

F Fisher's test $= kT + 1 = [1 + (k-1)\,r_I]/[1 - r_I]$

Intraclass correlation $= s_b^2/(s^2 + s_w^2) = T/T + 1$

r_k Coefficient of intraclass correlation based on k measurements

$$= (F-1)/F = T/\left(T + \frac{1}{k}\right) = kT/(kT+1)$$

$$= \frac{s^2}{s^2 + s_w^2/k}$$

e^{-m} The expected number in class 0 of the Poisson distribution

K Proportion of variance due to variation between measurements within the same patient $= 1/F = 1/(kT+1) = 1 - r_k$

k Number of measurements required by stated value of K or F and given T

$$= \left(\frac{1}{K} - 1\right)\frac{1}{T} = (F-1)\frac{1}{T}$$

$\bar{k}$ Average number of k in subsamples used in analyses of variance $= \left[Sk - \frac{Sk^2}{Sk}\right]\frac{1}{N-1}$

p Weight index for subsample mean based on k measurements $= (T+1)/\left(T + \frac{1}{k}\right) = 1/\mu^2_{\bar{x}k}$

μ Variance between subsample mean based on k measurements relative to variance based on one measurement per patient

$$= \left(T + \frac{1}{k}\right)/(T+1) = 1/p$$

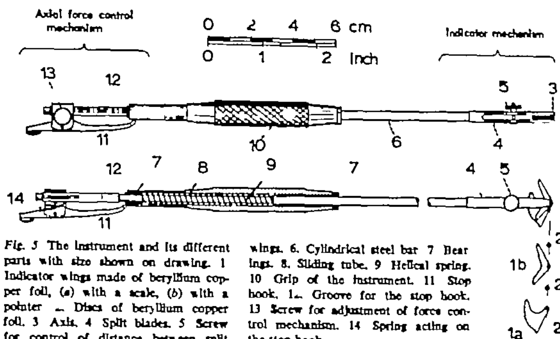


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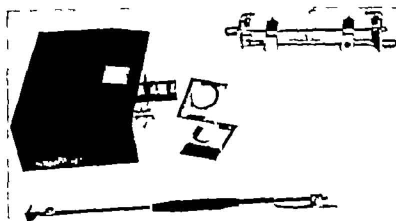


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For the pregnant women the mean number of measurements and the harmonic mean have been calculated, and the values recorded in early, middle and late pregnancy have been compared. The measurements which were performed after the parturition have been correlated to the values of the non-pregnant women.

The statistical symbols and equations used in this publication are listed below (8, 11, 19).

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k_i Number of measurements or number of children in subsample

x Measured value

M $\bar{x}$ Arithmetic mean of variates

Md Median (50 of f_e)

f Expected number or calculated number

f Observed number

d f Degrees of freedom

S(x) Sum of squared deviations

s^2 Variance = $S(x-\bar{x})^2$/d f

Standard deviation $\sqrt{s^2}$

C V Coefficient of variation in per cent 100 (s/M)

Student's t-test $= (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\bar{s}^2 \left(\frac{1}{k} \quad \frac{1}{k_2}\right)}$ or tabulated value of t

P Percentile point of frequency distribution

Skewness 3 (M Md)/

Standard error of the mean $s/\sqrt{k}$

$\bar{s}^2$ Pooled variance $\frac{S(\bar{x}_1 \quad x_1)^2 \quad S(\bar{x}_2 \quad x_2)^2}{k_1 \quad k_2 \quad 2}$

M Harmonic mean $= N/S\frac{1}{k}$

M $t_{s_{M}}$ 95% confidence interval

s^2_b Variance between patients

s^2 Variance within patients

T Ratio $= s^2_b/s^2$ $(F-1)/k$

F Fisher' test $= kT+1 = [1 \quad (k \quad 1)r]/1-$

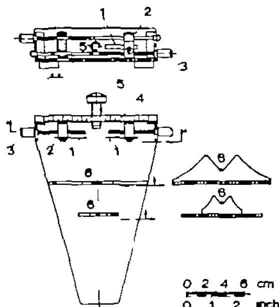


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Intraclass correlation $s^2_b/(s^2+s^2_b) = T/T+1$

Coefficient of intraclass correlation based on k measurements

$$=(F-1)/F = T/\left(T+\frac{1}{k}\right) = kT/(kT+1)$$

$$=\frac{s^2}{s^2+s^2_w/k}$$

e^{-m} The expected number in class 0 of the Poisson distribution

K Proportion of variance due to variation between measurements within the same patient $1/F = 1/(kT+1) = 1-$

k Number of measurements required by stated value of K or F and given T

$$=\left(\frac{1}{K}-1\right)\frac{1}{T} = (F \quad 1)\frac{1}{T}$$

k Average number of k in subsamples used in analyses of variance $= \left[Sk - \frac{Sk^2}{Sk}\right] \frac{1}{N \quad 1}$

p_T Weight index for subsample mean based on k measurements $= (T+1)/\left(T+\frac{1}{k}\right) = 1/\rho-$

s_T Variance between subsample mean based on k measurements relative to variance based on one measurement per patient

$$\left(T+\frac{1}{k}\right)/(T+1) = 1/\rho\, x\, k$$

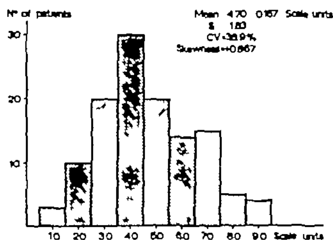


Fig 8 a. Frequency distribution of individual values in 30 women who were measured in 3 to 6 normal menstrual cycles.

M_w Weighted mean $= S(p\ k\ \bar{x})/S(p\ k)$

χ^2 Chi-square $= S[(f\ -f_c)^2/f_c]$

$t_{(\bar{x}\ \sqrt{M})}$ t test for the difference between and $\sqrt{M}$ in

$$\text{Poisson distribution} = \frac{\bar{x} - \sqrt{M}}{\sqrt{M/2N}}$$

$$s \quad \text{Standard error of} \quad = \frac{(1 - \ )\ [1 + (k-1)\ r)}{\sqrt{\tfrac{1}{2}k\ (k-1)\ N}}$$

RESULTS

1 *Analysis of variance of recorded values*

The values recorded on the instrument from 30 women who were *measured* in 3 to 6 normal menstrual cycles, are distributed as shown in Figs. 8 *a* and *b* and the result of the variance analysis is shown in Table IV The mean meas-

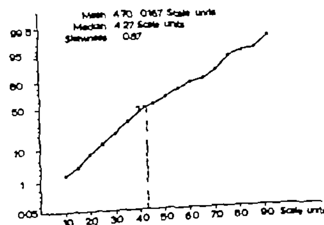


Fig 8 b Cumulative distribution of individual values in 30 women who were measured in 3 to 6 normal menstrual cycles.

Table IV *Analysis of variance of the measured values in 30 women who were monitored during 3 to 6 normal menstrual cycles*

$s = 1.833$, $k_0 = 4.022$, $T = 1.58$ and $s^2_k = 0.709$

Variation of values	Degrees of freedom	Sum of squares	Mean squares	F
Between patients	29	282.60	9.745	7.355
Within patients	91	120.62	1.325	$F > P_{.99}$
Total	120	403.22	3.360	

ured value in this group is $\bar{x} = 4.70 \pm 0.17$ scale units and the standard deviation $s = 1.833$ The *coefficient of variation* is C.V $= 38.9\%$ A highly significant difference between individual women in this group is demonstrated ($F = 7.355 > P_{.99}$) The intraclass correlation ($r = 0.612 \pm 0.083$) shows that the individual differences make up 61% of the total variance. The weighted mean is $M_w = 4.81$ and the variation about the individual mean is only $K = 0.136 = 13.6\%$ of the total variance. The variation between the individual means is $r_k = 0.864 = 86.4\%$ which shows that these measurements are highly characteristic for each woman and demonstrate individual differences.

2. *Measurements in non-pregnant women*

The results of the measurements ($k_i = 17$) performed on the 15 non-pregnant nulliparous women with normal menstrual cycles are as follows

Table V *Measurements performed on non-pregnant parous women with normal menstrual cycles*

No. of days in menstrual cycle	No. of measurements	$M \pm s_{\bar{x}}$		C.V
1 3	6	4.77 ± 0.20	0.50	10.5
4– 6	21	5.21 ± 0.50	2.28	43.8
7 9	21	5.36 ± 0.30	1.38	25.7
10–12	17	5.74 ± 0.49	01	35.0
13–15	14	4.98 ± 0.45	1.69	33.9
16–18	25	5.01 ± 0.37	1.83	36.5
19–21	18	4.74 ± 0.38	1.63	34.4
22 24	15	4.79 ± 0.48	1.86	38.8
25– 7	16	4.24 ± 0.40	1.59	37.5
28–30	9	4.14 ± 0.30	0.90	21.7
Sum and mea	162	4.97 ± 0.138	1.746	35.2



Fig. 9. Mean of measured values of cervical consistency at different time intervals in the menstrual cycle of 66 non-pregnant parous women (Table V).

$M \pm s_{\bar{x}}$ 4.23±0.41 scale units, t = 1.68 and C.V = 39.7%

Table V and Fig. 9 show the results obtained when the 66 non-pregnant parous women were measured (k_1 = 162). The mean measured value is higher than in the group above.

$M \pm s_{\bar{x}}$ 4.97±0.14 scale units, 1.746 and C.V = 35.2%

The number of women in each group varies and the number of measurements for the different women is not the same. It is therefore not surprising that the results show a wide distribution. There are no significant deviations from the general mean in each group, but a marked rise in the measured values is seen during the first 12 days of the menstrual cycle. During the last part of the cycle the curve falls until the onset of menstruation. The Student's *t*-test which has

Table VI. *Results of measurements on pregnant women*

No. of days before parturition	No. of measurements	$M \pm s_{\bar{x}}$		C.V
> 200	20	5.82±0.48	2.14	36.8
175–199	13	7.05±0.48	1.72	24.4
150–174	17	8.02±0.37	1.52	19.0
125–149	20	8.14±0.32	1.42	17.4
100–124	25	8.79±0.27	1.30	14.8
75–99	27	8.98±0.25	1.28	14.3
50–74	23	9.58±0.18	0.85	8.9
25–49	40	9.63±0.13	0.83	8.6
11–24	38	9.64±0.16	0.96	10.0
0–10	39	9.87±0.13	0.81	8.2

Table VII. *Measured values in pregnant women*

	Days before parturition	No. of measurements	$M \pm s_{\bar{x}}$		C.V
Nulliparous	150 and more	22	6.53±0.47	2.21	33.8
women	61–149	31	8.57±0.24	1.32	15.4
(N = 23)	0–60	54	9.69±0.12	0.90	9.3
Parous	150 and more	27	7.17±0.37	1.93	26.9
women	61–149	50	8.93±0.19	1.32	14.8
(N = 26)	0–60	76	9.68±0.10	0.88	9.1

been calculated for the non-pregnant nulliparous and parous women shows no significant difference between the measured values in the two groups (t = 1.66).

Nineteen women who were using the combined oral contraceptive (Table I and Fig. 2) have also been measured, and the measurements (k = 39) give the following result when the nulliparous and parous women are looked upon as one group:

$M \pm s_{\bar{x}}$ 5.35±0.37 scale units, = 2.29 and C.V = 42.8%

There is no significant difference between these women and the non-pregnant parous women (Table V). The *t*-test between the measured values of the non-pregnant nulliparous women and those using the combined oral contraceptive shows no significant difference (t = 1.82 and $P_{.05} < t < P_{.01}$)

3. *Measurements in pregnant women*

The results of measurements performed on the pregnant women are presented in Tables VI–VII, and in Figs. 10–11. For the nulliparous women the arithmetic mean number of measurements is $\bar{k}$ = 107/23 = 4.65 and the harmonic mean is M = 2.80. For the parous women the arithmetic mean number of measurements is $\bar{k}$ = 153/26 = 5.88 and the harmonic mean is M = 3.56.

As illustrated in Fig. 10 there is a rise in the measured values from early in pregnancy until delivery. At 200 days before parturition the mean of values recorded on the instrument was about 5.8 scale units. Five days before parturition the mean value was found to be about 9.9 scale units. The coefficient of variation was 36.8% at 200 days before term and 8.2% at 5 days before parturition. As illustrated in Fig. 11 there are sig

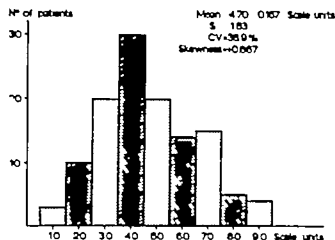


Fig 8 a. Frequency distribution of individual values in 30 women who were measured in 3 to 6 normal menstrual cycles.

M_w Weighted mean $= S(p\ x\ \bar{x})/S(p\ x)$

χ Chi-square $= S[(f - f_c)\ /f_c]$

$t_{(\ \sqrt{M})}$ *t*-test for the difference between *s* and $\sqrt{M}$ in

$$\text{Poisson distribution} = \frac{s - \sqrt{M}}{\sqrt{M/\ N}}$$

$$s \quad \text{Standard error of } r = \frac{(1-\ )\ [1+(k-1)\ r]}{\sqrt{\tfrac{1}{2}k\ (k-1)\ N}}$$

RESULTS

1 *Analysis of variance of recorded values*

The values recorded on the instrument from 30 women who were measured in 3 to 6 normal menstrual cycles, are distributed as shown in Figs. 8 *a* and *b* and the result of the variance analysis is shown in Table IV The mean measured value in this group is $\bar{x} = 4.70 \pm 0.17$ scale units and the standard deviation $s = 1.833$. The coefficient of variation is C.V $= 38.9\%$ A highly significant difference between individual women in this group is demonstrated ($F = 7.355 > P_{.99}$) The intraclass correlation ($r = 0.612 \pm 0.083$) shows that the individual differences make up 61% of the total variance. The weighted mean is $M_w = 4.81$ and the variation about the individual mean is only $K = 0.136 = 13.6\%$ of the total variance. The variation between the individual means is $r_L = 0.864 = 86.4\%$ which shows that these measurements are highly characteristic for each woman and demonstrate individual differences.

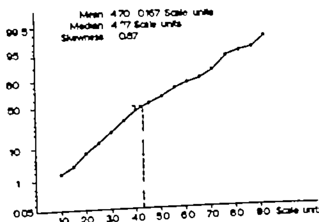


Fig 8 b. Cumulative distribution of individual values in 30 women who were measured in 3 to 6 normal menstrual cycles.

Table IV *Analysis of variance of the measured values in 30 women who were monitored during 3 to 6 normal menstrual cycles*

$s = 1.833$, $k_0 = 4.022$, $T = 1.58$ and $s^2_L = 0.709$

Variation of values	Degrees of freedom	Sum of squares	Mean squares	F
Between patients	29	[illegible]82.60	9.745	7.335
Within patients	91	120.62	1.3.5	$F > P_{.99}$
Total	120	403.2[illegible]	3.360	

2. *Measurements in non-pregnant women*

The results of the measurements ($k_1 = 17$) performed on the 15 non-pregnant nulliparous women with normal menstrual cycles are as follows.

Table V *Measurements performed on non-pregnant parous women with normal menstrual cycles*

No. of days in menstrual cycle	No of measurements	$M \pm s\bar{x}$		CV
1 3	6	4 77±0.20	0 50	10 5
4– 6	1	5 21±0 50	2.78	43.8
7 9	21	5 36±0 30	1 38	[illegible]5 7
10–12	17	5 74±0 49	2.01	35 0
13–15	14	4 98±0 45	1 69	33 9
16–18	[illegible]5	5.01±0 37	1 83	36 5
19–21	18	4 74±0.38	1 63	34 4
[illegible] 24	15	4 79±0 48	1 86	38.8
25– 7	16	4 4±0.40	1 59	37.5
28–30	9	4 14±0.30	0 90	21 7
Sum and mea	162	4 97±0.118	1 746	35.2



Fig. 9. Mean of measured values of cervical consistency at different time intervals in the menstrual cycle of 66 non-pregnant parous women (Table V).

$M \pm s_{\bar{x}}$ 4.23±0.41 scale units, –1.68 and C.V. = 39.7%

Table V and Fig. 9 show the results obtained when the 66 non-pregnant parous women were measured (k_1 = 162). The mean measured value is higher than in the group above:

$M \pm s$ 4.97±0.14 scale units, 1.746 and C.V. = 35.2%

The number of women in each group varies and the number of measurements *for the different* women is not the same. It is therefore not surprising that the results show a wide distribution. There are no significant deviations from the general mean in each group, but a marked rise in the measured values is seen during the first 12 days of the menstrual cycle. During the last part of the cycle the curve falls until the onset of menstruation. The Student's *t*-test which has

Table VI. *Results of measurements on pregnant women*

No. of days before parturition	No. of measurements	$M \pm s_{\bar{x}}$		C.V.
> 200	20	5.82±0.48	2.14	36.8
175–199	13	7.05±0.48	1.72	24.4
150–174	17	8.02±0.37	1.52	19.0
125–149	20	8.14±0.32	1.42	17.4
100–124	23	8.79±0.27	1.30	14.8
75–99	27	8.96±0.25	1.28	14.3
50–74	23	9.38±0.18	0.85	8.9
25–49	40	9.63±0.13	0.83	8.6
11–24	38	9.64±0.16	0.96	10.0
0–10	39	9.87±0.13	0.81	8.2

Table VII. *Measured values in pregnant women*

	Days before parturition	No. of measurements	$M \pm s$		C.V.
Nulli-parous women (N = 23)	150 and more	22	6.53±0.47	2.21	33.8
	61–149	31	8.57±0.24	1.32	15.4
	0–60	54	9.69±0.12	0.90	9.3
Parous women (N = 26)	150 and more	27	7.17±0.37	1.93	26.9
	61–149	50	8.93±0.19	1.32	14.8
	0–60	76	9.68±0.10	0.88	9.1

been calculated for the non-pregnant nulliparous and parous women shows no significant difference between the measured values in the two groups (t = 1.66).

Nineteen women who were using the combined oral contraceptive (Table I and Fig. 2) have also been measured, and the measurements (k_1 = 39) give the following result when the nulliparous and parous women are looked upon as one group:

$M \pm s_{\bar{x}}$ 5.35±0.37 scale units, s 2.29 and C.V. = 42.8%

There is no significant difference between these women and the non-pregnant parous women (Table V). The *t*-test between the measured values of the non-pregnant nulliparous women and those using the combined oral contraceptive shows no significant difference (t = 1.82 and $P_{.05} < t < P_{.02}$).

3. *Measurements in pregnant women*

The results of *measurements performed* on the pregnant women are presented in Tables VI–VII, and in Figs. 10–11. For the nulliparous women the arithmetic mean number of measurements is $\bar{k}$ = 107/23 = 4.65 and the harmonic mean is M = 2.80. For the parous women the arithmetic mean number of measurements is $\bar{k}$ = 153/26 = 5.88 and the harmonic mean is M = 3.56.

As illustrated in Fig. 10 there is a rise in the measured values from early in pregnancy until delivery. At 200 days before parturition the mean of values recorded on the instrument was about 5.8 scale units. Five days before parturition the mean value was found to be about 9.9 scale units. The coefficient of variation was 36.8% at 200 days before term and 8.2% at 5 days before parturition. As illustrated in Fig. 11 there are sig-

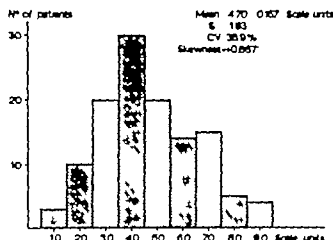


Fig 8 a. Frequency distribution of individual values in 30 women who were measured in 3 to 6 normal menstrual cycles.

M_w Weighted mean $= S(p\ k\ \bar{x})/S(p\ k)$

χ^2 Chi-square $= S[(f - f_e)^2/f_e]$

$t_{(s-\sqrt{M})}$ t-test for the difference between s and $\sqrt{M}$ in

$$\text{Poisson distribution} = \frac{s - \sqrt{M}}{\sqrt{M/2N}}$$

$$\text{Standard error of } = \frac{(1-r)\ \{1+(k-1)\ r\}}{\sqrt{\tfrac{1}{2}k\ (k-1)\ N}}$$

RESULTS

1 *Analysis of variance of recorded values*

The values recorded on the instrument from 30 women who were measured in 3 to 6 normal menstrual cycles, are distributed as shown in Figs. 8 *a* and *b* and the result of the variance analysis is shown in Table IV The mean measured value in this group is $\bar{x} = 4.70 \pm 0\ 17$ scale units and the standard deviation $s = 1\ 833$. The coefficient of variation is CV = 38.9 % A highly significant difference between individual women in this group is demonstrated ($F = 7.355 > P_{.99}$) The intraclass correlation ($r = 0.61? \pm 0.083$) shows that the individual differences make up 61 % of the total variance. The weighted mean is $M_w = 4\ 81$ and the variation about the individual mean is only $\lambda = 0\ 136 = 13\ 6$ % of the total variance. The variation between the individual means is $r_L = 0\ 864 = 86.4$ % which shows that these measurements are highly characteristic for each woman and demonstrate individual differences.

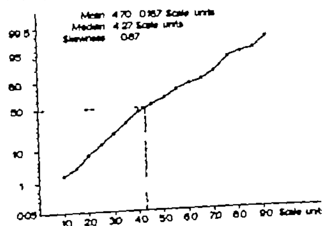


Fig 8 b. Cumulative distribution of individual values in 30 women who were measured in 3 to 6 normal menstrual cycles.

Table IV *Analysis of variance of the measured values in 30 women who were monitored during 3 to 6 normal menstrual cycles*

$= 1.833$, $k_0 = 4.022$, $T = 1.58$ and $^2_k = 0.709$

Variation of values	Degrees of freedom	Sum of squares	Mean squares	F
Between patients	29	282.60	9 745	7.355
Within patients	91	120.62	1.325	$F > P_{.99}$
Total	120	403.22	3.360	

2 *Measurements in non-pregnant women*

The results of the measurements ($k_i = 17$) performed on the 15 non-pregnant nulliparous women with normal menstrual cycles are as follows.

Table V *Measurements performed on non-pregnant parous women with normal menstrual cycles*

No. of days in menstrual cycle	No. of measurements	$M \pm s$		CV
1 3	6	4.77 ± 0.20	0 50	10 5
4– 6	21	5 21 ± 0.50	2	43.8
7 9	21	5 36 ± 0 30	1 38	43.7
10–12	17	5 74 ± 0 49	2 01	35 0
13–15	14	4 98 ± 0 45	1 69	33 9
16–18	25	5 01 ± 0.37	1 83	36 5
19–21	18	4.74 ± 0.38	1.63	34 4
2. 24	15	4 79 ± 0 48	1.86	38 8
25–27	16	4 24 ± 0.40	1.59	37.5
28–30	9	4 14 ± 0 30	0 90	21 7
Sum and mean	162	4 97 ± 0 138	1 746	35.2

Table VIII. *Results of measurements on the uterine cervix after parturition*

No of days after parturition	No of measurements	$M \pm \sigma_{\bar{x}}$		CV
(14–54) 36.6	21	6.43 ± 0.39	1.77	27.5
(57–299) 93.2	24	6.15 ± 0.33	1.62	26.3

the menstrual cycle the oestrogenic stimulation causes thickening of the vaginal epithelium (18). This epithelium is also a target structure for the hormone effects in women using a combined oral contraceptive, and individual differences depending on response and dosage is likely to occur. The body weight may also be of importance. During pregnancy the squamous epithelium reveals thickening of the superficial layers (17).

The cervical stroma with all its components is the tissue immediately beneath the basement membrane of the epithelium. It is obvious that when 20 g force is applied during the measurement, the stroma must exert some resistance against the impression. The cervix is described as gaining in size and vascularity under the influence of oestrogens (18), and the hormonal fluctuations occurring in menstrual cycle, in pregnancy and when oral contraception is being used, are therefore of importance to the consistency.

Scars after parturition, infection of the cervical glands and individual anatomic differences are probably the reason for the great variation in the recorded values. Free nerve endings have been demonstrated in the papillae of the stratified epithelium of the cervix, and contractions of muscle fibres might take place when the epithelium is touched by the impressed instrument. Thus the consistency could appear less soft. In pregnancy contractions of the uterine musculature are known to take place (Braxton Hicks sign), and it is reasonable to believe that the cervical "tonus" is influenced by these contractions.

During measurement, the instrument was in contact with the uterine cervix for about 2 seconds only. Nevertheless, it is possible that some secretion from the cervix may penetrate the indicator mechanism of the instrument. This may happen even if the discharge is wiped out of the vagina before the measurement. The capillary forces acting between the indicator wings and the discs on each side are supposed to absorb the fluid. These effects do not disturb the first measurement, but if another measurement is performed shortly thereafter a different value may result, depending on the type of vaginal secretion. If the fluid is mucinous, the indicator wings may rotate more easily and a higher recorded value is the result. A more adhesive fluid, on the other hand, causes greater friction between the wings and a lower reading will result. The capillary forces may also have another effect in that the indicator wings are pressed together from side to side when the small spaces in the indicator mechanism are filled with fluid, since the distance between the indicator blades is the same (see Fig. 5).

The present experiments show that variation in the cervical consistency does take place during the menstrual cycle. A maximum degree of softness is recorded when the preovulatoric oestrogen peak is usually seen. Individual differences are found, but the mean value recorded in the normal cycle is lower than the mean value for the women using the combined oral contraceptive. It is possible that the oestrogenic component in the contraceptive pill is responsible for this.

If a woman has been measured with the instrument before she has become pregnant, so that the values for her normal menstrual cycle are known, the measurements at about 12 weeks of pregnancy will show definitely higher values. This happened to be the case in three of the women in the series and the measurements contributed to the diagnosis.

The mean measured value for parous pregnant women is higher than that in primigravidae, but the difference is not significant (Fig. 11). From a clinical point of view the parous cervix was expected to be the softest.

CONCLUDING REMARKS

It must be noted that the maximum reading of the instrument in the present design is 10.5 scale units. In some pregnancies the cervix may be too soft to obtain a recording on the indicator mechanism. This can happen if the pregnant woman is near term. As seen in Fig. 10, the measured curve rises in the last 2 weeks before parturition.

It is therefore desirable to make some improvements to the instrument, and some relevant sug-

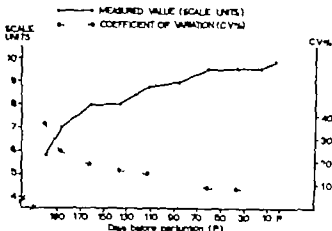


Fig 10 Measured values of cervical consistency during pregnancy and coefficient of variation.

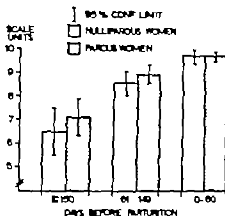


Fig 11 Measured values of cervical consistency at 3 different time intervals in pregnancy (Table VII).

nificant differences between the recorded values in the different time intervals during pregnancy

The calculations shown in Table VII do not demonstrate any significant differences between the nulliparous and parous pregnant women.

The recordings concerning one woman who developed toxemia in pregnancy and another women who had a twin pregnancy (Fig. 12) show no deviations from the normal group (Fig. 10–11).

Table VIII shows the results obtained when the measurements were performed up to 259 days post partum. The 35 women with 45 measurements were divided into two groups, during the first 8 post partum weeks 21 measurements were performed, and 24 measurements subsequently Only 6 measurements were made after 100 days post partum. Table VIII illustrates a marked decrease of the mean measured value in the post partum period. The mean value approaches the normal values for non-pregnant women, but the value 4 97 scale units (see Table V) is not reached even at 93 days after the parturition.

DISCUSSION

When the indicator end of the instrument is pressed lightly against the anterior lip of the uterine cervix the instrument at first depresses the squamous epithelium to some extent. Resistance against the applied force must therefore partly be caused by this epithelium and its basement membrane The thickness and liquid content of the epithelial cells and the flexibility of the basement membrane must be of importance. In

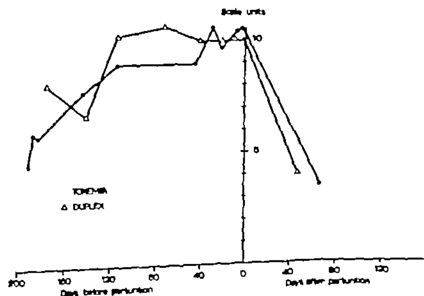


Fig 1 Values of measurements in 2 patients who were monitored during pregnancy and after parturition (see text).

Table VIII. *Results of measurements on the uterine cervix after parturition*

No of days after parturition	No. of measurements	$M \pm s_M$		C.V
(34-56) 36.6	21	6.43 ± 0.39	1.77	27.5
(57-239) 93.2	24	6.15 ± 0.33	1.62	26.3

the menstrual cycle the oestrogenic stimulation causes thickening of the vaginal epithelium (18). This epithelium is also a target structure for the hormone effects in women using a combined oral contraceptive, and individual differences depending on response and dosage is likely to occur. The body weight may also be of importance. During pregnancy the squamous epithelium reveals thickening of the superficial layers (17).

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Scars after parturition, infection of the cervical glands and individual anatomic differences are probably the reason for the great variation in the recorded values. Free nerve endings have been demonstrated in the papillae of the stratified epithelium of the cervix, and contractions of muscle fibres might take place when the epithelium is touched by the impressed instrument. Thus the consistency could appear less soft. In pregnancy contractions of the uterine musculature are known to take place (Braxton Hicks sign), and it is reasonable to believe that the cervical tissue is influenced by these contractions.

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It is therefore desirable to make some improvements to the instrument, and some relevant sug-

gestions have been made in the report on the instrument. When such improvements have been carried out, it will be of interest to analyse whether there is any correlation between early softening of the cervix in pregnancy and short duration of labour A hard leathery and un dilatable cervix on the other hand, is described to be a cause of cervical dystocia which causes prolonged labour (9) It is also of interest to ex amine whether a high value may be helpful in predicting the date of expected delivery

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REFERENCES

1 Bakke, T A new mechanical instrument for the measurement of fibro-elasticity with special reference to its use in the assessment of the consistency of the uterine cervix. Acta Obstet Gynecol Scand *52* 77 1973
2. Berwind, T Electronenmikroskopische Untersuchungen am Fasersystem der Cervix uteri der Fra Arch Gynäk *184* 459 1954
3 Buckingham, J C., Selden, R. & Danforth, D N Connective tissue changes in the cervix during preg nancy and labor Ann NY Acad Sci *97* 733 1962.
4 Cretius, K *I* Gynakologie und Geburtshilfe, Band II (ed. O Käser V Friedberg, K. G Ober K. Thomsen & J Zander) p 139 Georg Thieme, Stuttgart, 1967
5 Danforth, D N The fibrous nature of the human cervix, and its relation to the isthmic segment in gravid and nongra id uteri. Amer J Obstet Gynecol *53* 541 1947
6 Davies, D V & Coupland, R E.. Gray's Anatomy 34th edn p. 953 Longmans, Green & Co Ltd, London and Harlow 1967
7 Da ies, J *I* Textbook of Obstetrics and Gynecology (ed. D N Danforth), 2nd edn, p. 99 Harper & Row, New York, Evanston, San Francisco and London, 1971
8 Dixon, J W & Massey J F Jr Introduction to Statistical Analysis, 2nd edn. McGraw-Hill Book Company Inc., New York, Toronto and London, 1957
9 Donald, I.. Practical Obstetric Problems, 4th edn, p. 511 Lloyd-Luke, London, 1972.
10 Dougherty C. M. & Low F M.. The fine structure of the basement membrane of the uterine cervical epithelia. Amer J Obstet Gynecol *76* 839 1958.
11 Fisher R. A Statistical Methods for Research Workers, 11th edn. Oliver & Boyd, Edinburgh and London, 1950
12. Fluhmann, C. F *I* Obstetrics (ed. J P Greenhill, 13th edn, p. 187 W B. Saunders, Philadelphia and London, 1965
13 Hall, R., Anderson, J & Smart, G A.. Fundamentals of Clinical Endocrinology p. 152. Pitman, London, 1969
14 Krantz, K. E. Innervation of the human uterus. Ann NY Acad Sci *75* 770 1959
15 Krantz, K. E. & Phillips, W P Anatomy of the human uterine cervix, gross and microscopic. Ann NY Acad Sci *97* 551 1962.
16 Llewellyn-Jones, D Fundamentals of Obstetrics and Gynecology vol I Obstetrics. F ber & Faber London, 1971
17 Murphy E. J & Herbut, P A.. The uterine cervix during pregnancy Amer J Obstet Gynecol *59* 384, 1950
18. Ryan, K. J *I* Principles and Management of Human Reproduction (ed. D E. Reid, K. J Ryan & K. Benirschke) p. 13 and 99 W B Saunders, Philadelphia and Toronto 1972.
19 Wright, S. Genetic and Biometric Foundations, ol I The University of Chicago Press, Chicago and London, 1968.

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GONADOTROPHIN RESPONSE TO LUTEINIZING HORMONE RELEASING FACTOR (LRF) IN PUERPERAL WOMEN

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Abstract. Synthetic luteinizing hormone releasing factor (LRF) was administered intravenously in a dose of 200 µg to twenty normal lactating puerperal women and serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) response to LRF was measured by double antibody radio-immunoassay. Synthetic LRF failed to stimulate FSH secretion in all the eight volunteers in the 1st postpartum week. About half of the six subjects in the 3rd postpartum week responded to LRF with a rise of serum FSH and LH. All the six lactating women in the 5th postpartum week were responsive to LRF and there was a concomitant rise in serum FSH and LH. These results suggest that gonadotrophic activity of the anterior pituitary is suppressed by an unknown mechanism during the first few weeks of puerperium and gonadotrophin reserve function recovers completely around the 5th postpartum week. Thus, it might be assumed that puerperal anovulation or amenorrhoea is due to hypothalamic-pituitary dysfunction during the first few postpartum weeks and due to hypothalamic disorders after the 5th postpartum week.

The gonadotrophic function of the anterior pituitary during the postpartum period is still obscure. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels during the postpartum period have been measured using radio-immunoassay methods by several investigators (2, 3, 4, 12), but little information is available about gonadotrophin reserve function of the hypophyseal gland during the postpartum period.

In 1971 luteinizing hormone releasing factor (LRF) was isolated from porcine hypothalami and its structure was elucidated by Schally's group (1, 5, 13, 14). The decapeptide was soon synthesized (6) and this synthetic LRF was effective in evoking release of both FSH and LH from the pituitary in human subjects (8, 9, 11, 14, 15). These observations demonstrated the potential value of LRF in the clinical evaluation of the capacity of the pituitary to synthesize and release FSH and LH. In order to elucidate gonadotrophin reserve function of the anterior pituitary during the postpartum period, the response to synthetic LRF in normal lactating puerperal women was investigated.

The purpose of the present communication is to report serum FSH and LH concentrations and response to LRF in normal lactating puerperal women and to discuss the gonadotrophic function of the hypophyseal gland during the postpartum period.

MATERIALS AND METHODS

Synthetic LRF (MO-1208) used in this study was kindly supplied by Mochida Pharmaceutical Company, Tokyo, Japan. The structure of this LRF and the results of toxicity study were described elsewhere (9).

Volunteers

Twenty normal lactating puerperal women were given synthetic LRF. These twenty volunteers ranged in age from 24 to 36 years and had had uneventful pregnancies and deliveries. Eight volunteers were in the 1st postpartum week, six were in the 3rd and six were in the 5th postpartum week. All the volunteers were lactating. Consent was obtained from each subject after full explanation of the purpose and nature of this investigation.

LRF administration

In terms of the dosage of LRF employed in our previous clinical investigation a dose of 200 µg was injected intravenously over 30 sec in each subject. Venous blood samples were obtained before the injection and additional samples at 15, 30, 45, 60 and 120 min after the be-

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Table I *Effect of LRF on the serum concentration of FSH in puerperal women in the first postpartum week*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of FSH in mIU 2nd IRP-HMG/ml serum					
		0	15	30	45	60	120
Subject	Day of puerperium						
M M	4	2.1	2 9	2.8	4 6	3 0	2.4
F K	4	2.5	2.8	3 0	.6	2.5	2.6
S T	4	2.0	3 1	3 4	2.6	2 4	.5
K. K.	4	3 0	3.6	4 1	2.8	.8	2.8
S M	4	2.1	2.6	2.8	2.2	2.4	2.6
S K.	4	.0	2.8	2.1	2 2	2.4	2.3
I H	4	2.8	2.8	2.5	2.4	2.6	2.0
E. Y	4	3 4	3 2	3 6	4 0	4 0	4 4

ginning of the injection, for determination of serum FSH and LH concentrations

Immuno-reactive FSH and LH levels were assayed in duplicate by double antibody radio-immunoassay methods modified from those of Midgley (7) and Odell et al (10) The standard preparations were the second international reference preparation of human menopausal gonadotrophin (2nd IRP-HMG) and LER 907 The average relative potencies of these preparations were 49 IU of 2nd IRP-HMG/mg LER 907 for FSH and 304 IU of 2nd IRP-HMG/mg LER 907 for LH The results of this study were expressed as mIU of 2nd IRP-HMG/ml serum.

RESULTS

The serum FSH response to LRF in puerperal women in the 1st postpartum week is shown in

Table II *Effect of LRF on the serum concentration of FSH in puerperal women in the 3rd postpartum week*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of FSH in mIU 2nd IRP-HMG/ml serum					
		0	15	30	45	60	120
Subject	Day of puerperium						
Y N	15	3 0	3 0	3.5	3 2	3.	3.8
A H	17	3.6	5 9	9 0	10.6	12 0	11 6
E I	18	9.8	15 4	19.5	19 7	18.5	20 0
T Y	20	12.0	20.2	24.3	.3 6	27.2	21.5
H O	21	18.3	53.2	57 1	84 0	77.2	52.1
R. I	21	18.0	40.2	57.3	60.2	59.3	56.2

Table III *Effect of LRF on the serum concentration of LH in puerperal women in the 3rd postpartum week*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of LH in 2nd IRP-HMG/ml serum					
		0	15	30	45	60	120
Subject	Day of puerperium						
Y N	15	11.2	12.6	12.0	10.6	10 0	9.1
A H	17	11 4	11 4	15.	11.6	12.0	8.9
E. I	18	15 4	18.3	23.6	18 4	19.2	16.1
T Y	20	6.1	8 6	6.7	8.7	9 0	8.5
H O	21	6.2	103.2	13.0	153.6	76 1	71.6
R I	21	8.6	55 4	73 4	52.	54 0	32.2

Table I Serum FSH concentrations before LRF administration were low but detectable Synthetic LRF failed to stimulate FSH secretion in all the eight volunteers in the 1st postpartum week and serum FSH levels remained still low after LRF injection (Table I)

The serum FSH and LH response to LRF in subjects in the 3rd postpartum week was shown in Tables II and III and Figs 1 and 2 Serum FSH levels were relatively low compared with normal menstrual cycle levels Four subjects responded to synthetic LRF with a rise of serum FSH concentration but another two did not (Table II and Fig. 1) Serum LH levels were also relatively low Three subjects were responsive to LRF with an elevation of serum LH Three showed no response (Table III and Fig 2)

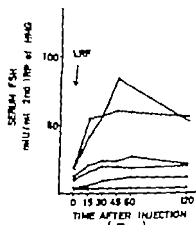
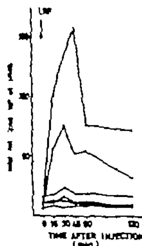


Fig 1 Effect of LRF on the serum concentration of FSH in puerperal women in the 3rd postpartum week.



ig 2. Effect of LRF on the serum concentration of H in puerperal women in the 3rd postpartum week.

Table IV *Effect of LRF on the serum concentration of FSH in puerperal women in the 5th post partum week*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of FSH in mIU 2nd IRP-HMG/ml serum					
		0	15	30	45	60	120
Subject	Day of puerperium						
Y. H.	28	8.6	11.2	20.5	52.8	26.7	26.7
S. K.	29	9.3	29.5	35.0	47.8	39.6	36.3
E. W	30	7.3	33.2	40.9	47.0	42.9	30.0
K. K.	30	6.0	26.2	33.3	50.2	33.0	33.0
S. O	30	8.3	25.2	42.0	46.4	43.2	36.5
A. T	31	8.6	27.3	40.0	46.2	39.3	43.2

The gonadotrophin response to LRF in women at the 5th postpartum week was illustrated in Tables IV and V and Figs. 3 and 4. Serum FSH and LH concentrations were in a lower range of less seen in the normal menstrual cycles. There was a clear rise of serum FSH and LH in all instances (Tables IV and V and Figs 3 and 4).

The pattern and magnitude of response in these puerperal women were approximately analogous to loss seen in normal females of reproductive age.

No serious side effects of LRF were noted and the postpartum course of all these volunteers was uneventful.

DISCUSSION

The gonadotrophic function of the anterior pituitary during the postpartum period remains in doubt and little is known about gonadotrophin reserve function of the hypophyseal gland. The cause of puerperal anovulation and amenorrhoea is quite obscure.

Fukman et al. (3) and Saxena et al. (12) using radio-immunoassay methods found normal follicular phase levels of serum FSH and LH at the 6th postpartum week. Jaffe et al. (4) reported low levels of serum FSH in one postpartum patient who had been studied daily until the 22nd postpartum day when rise occurred to follicular phase levels while LH returned to follicular phase levels after the clearance of human chorionic gonadotrophin (HCG). Likewise Crystle et al. (2) reported detectable, but low levels of serum FSH and LH for lactating puerperal women.

In this series serum FSH levels for puerperal women were detectable, but relatively low in the 1st postpartum week. Moreover no response to exogenously administered LRF was demonstrated and there was no rise in serum FSH levels at all. Serum FSH levels returned to the normal follicular phase levels in four of the six subjects in the 3rd postpartum week, and these four subjects were responsive to LRF with a significant rise of serum FSH. In contrast, serum LH levels were relatively low but three of the six subjects responded to LRF with a rise of serum LH. All the six volunteers

Table V *Effect of LRF on the serum concentration of LH in puerperal women in the 5th postpartum week*

Time in minutes after intravenous injection of 200 μg LRF		Serum concentration of LH in mIU 2nd IRP HMG/ml serum					
		0	15	30	45	60	120
Subject	Day of puerperium						
Y. H.	28	8.6	30.3	17.2	24.8	23.8	17.8
S. K.	29	7.9	64.0	67.3	64.0	66..	33.5
E. W	30	10.9	185.5	240.3	194.2	148.5	145.0
K. K	30	12.2	78.0	112.5	119.0	99.6	72.3
S. O	30	12.2	49.3	53.2	56.2	42.9	41.2
A. T	31	16.3	53.0	59.0	54.0	47.0	36.0

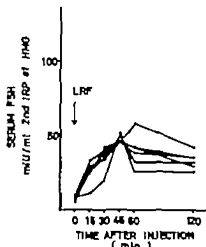


Fig 3 Effect of LRF on the serum concentration of FSH in puerperal women in the 5th postpartum week.

in the 5th postpartum week responded to LRF with a concomitant rise of serum FSH and LH

These results suggest that gonadotrophic function of the hypophyseal gland is suppressed by an unknown mechanism during the first few post partum weeks It is assumed that the return of normal gonadotrophic activity of the anterior pituitary begins around the 3rd postpartum week and gonadotrophin reserve function recovers completely around the 5th postpartum week. Thus it might be concluded that puerperal anovulation and amenorrhoea is due to hypothalamic-pituitary dysfunction during the first few weeks of puerperium and due to hypothalamic disorders after the 5th postpartum week

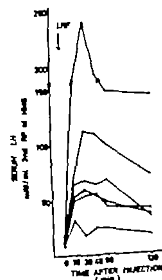


Fig 4 Effect of LRF on the serum concentration of LH in puerperal women in the 5th postpartum week.

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REFERENCES

1 Baba, Y Matsuo H. & Schally A. V Structure porcine LH and FSH-releasing hormone II Con mation of the proposed structure by conventio sequential analysis Biochem Biophys Res Comm 459 1971
2. Crystle C. D Powell J & Stevens, V C., Plas gonadotrophins during the postpartum period (stet & Gynecol 36 887 1970
3 Faiman, C Ryan R. J Zwirek, S J & Robin, M. Serum FSH and HCG during human pregnancy a puerperium J Clin Endocrinol 28 1323 1968.
4 Jaffe, R. B Lee P A & Midgley A R., Ser gonadotropins before, at the inception and follow human pregnancy J Clin Endocrinol 29 1281 19
5 Matsuo, H Baba, Y Nair R M G Arimura, & Schally A V Structure of the porcine LH a FSH-releasing hormone. I Proposed amino a sequence. Biochem Biophys Res Commun 43 13 1971
6 Matsuo H. Arimura, A. Nair R. M G & Schal A V The synthesis of porcine LH and FSH-rele ing hormone by the solid phase method. Bioch Biophys Res Commun 45 654 1971
7 Midgley A R. Jr Radioimmunoassay of human f licle-stimulating hormone J Clin Endocrinol 2 295 1967
8 Nakano R. Kotsuji, F Mizuno T Hashiba, Washio M & Tojo S Response to luteinizing h mone releasing factor (LRF) in normal subjects a anovulatory patients. Acta Obstet Gynecol Sca 52 171 1973
9 Nakano R. Mizuno T Kotsuji F Katayam K. Washio M & Tojo, S Triggering of ovu tion after infusion of synthetic luteinizing hormo releasing factor (LRF) Acta Obstet Gynecol Sca 52 269 1973
10. Odell W D Rayford, P L. & Ross G T Simp fied, partially automated method for radioimmun assay of human thyroid-stimulating, growth, lutein ing and follicle stimulating hormones J Lab Cl Med 70 973 1967
11 Rebar R. Yen S S C Vandenberg, G Naftoli F Ehara, Y Engblom S. Ryan R. J Rivier J Amoss M & Guillemin R. Gonadotropin respons to synthetic LRF Dose-response relationship in me J Clin Endocrinol 36 10, 1973
12 Saxena, B B Gandy H M & Peterson, R E "Gonadotropins 1968 (ed. E Rosemberg), p. 33 Geron-X Inc Los Altos Calif 1968.

13. Schally A. V Baba, Y Arimura, A., Redding, T. W & White W. F Evidence for peptide nature of LH and FSH-releasing hormones. Biochem Biophys Res Commun 42 50 1971
14. Schally A. V., Kastin, A. J & Arimura, A. Hypothalamic FSH- and LH-regulating hormone: Structure, physiology and clinical studies. Fertil Steril 22 703 1971
15. Yen, S. S. C., Rebar R., Vandenberg, G., Naftolin, F Ehara, Y Engblom, S., Ryan, K. J Benirschke, K., Rivier J., Amoss, M. & Guillemin, R. Synthetic luteinizing hormone releasing factor: A potent stimulator of gonadotropin release in man. J Clin Endocrinol 34 1108 1972.

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FETAL BRAIN DAMAGE FOLLOWING MATERNAL CARBON MONOXIDE INTOXICATION AN EXPERIMENTAL STUDY

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Abstract. Techniques of fetal monitoring, including fetal blood sampling *in utero* were employed to study the physiological effects of acute maternal carbon monoxide intoxication upon the fetal rhesus monkey. Nine term-pregnant female monkeys were exposed to 0.1–0.3 % inspired CO over 1–3 hours. The mothers tolerated carboxyhemoglobin (COHb) levels exceeding 60 % without clinical sequelae. The fetuses promptly developed profound hypoxia upon exposure of the mothers to CO However the fetal COHb levels rose only gradually over the 1–3 hours and thus contributed only slightly to the development of the early fetal hypoxia. Fetal hypoxia was associated with bradycardia, hypotension, and metabolic and, later respiratory acidosis A close correlation was noted between the severity of intrauterine hypoxia and the appearance of brain damage. Severe brain damage (brain swelling associated with hemorrhagic necrosis of the cerebral hemispheres) appeared only in those fetuses whose arterial O_2 content had fallen below 2.0 ml/100 ml for at least 45 min during the maternal CO intoxication

In view of the hazards of carbon monoxide gas as an environmental pollutant (8) and a potentially lethal intoxicant understanding of its disease producing potential under diverse clinical circumstances is important. Since Breslau original description of illuminating gas intoxication of pregnant mothers (3), over 24 additional cases of carbon monoxide poisoning during pregnancy have been reported (1 11) Although the mother died in only one-third of these cases, the fetus succumbed in over two-thirds (5). Depending upon gestational age at the time of intoxication, the surviving infant have exhibited severe mental retardation, motor disabilities involuntary movements hydrocephalus and variety of anomalies of development. The number of cases vailable for study however has been too small to permit a meaningful analysis of the incidences of the various types of pathological changes observed in the human (13)

A number of studies (5 11) have established that CO does indeed pass into the fetal circulation and that higher levels of fetal carboxyhemoglobin are associated with increased risk of fetal death. Nonetheless, information is lacking as to the detailed pathophysiology of maternal-fetal CO intoxication and particularly of the determinants of fetal mortality and morbidity The present study attempts to develop such information using the rhesus monkey which both in its central nervous system and its placenta is closely similar to man (12, 15).

METHODS

Nine pregnant rhesus monkeys with near-term pregnancies were used. Eight with dated pregnancies were studied on days 156–159 of gestation. The ninth the only undated pregnancy was judged to be near term by maternal abdominal palpation (6).

Following parenteral sodium pentobarbital anesthesia, 15 mg/kg, maternal endotracheal intubation and femoral artery catheterization were carried out. A hysterotomy was performed, the left fetal leg was exteriorized, and polyethylene catheter was introduced into the left fetal femoral artery Subcutaneous electrocardiographic leads were also secured in place on the fetus After all fetal parts were replaced into the uterus, the end of second catheter was secured within the uterine cavity and the maternal uterine and abdominal wall incisions were closed permitting the free ends of all catheters and leads to pass to the outside Amniotic fluid lost during the procedure was replaced with 40 ml of physiological saline. These techniques are reported in greater detail elsewhere (14).

The maternal and fetal arterial blood pressures and

heart rates and the intrauterine pressure were recorded continuously on an 8-channel polygraph (Brush Instruments Gould, Inc.) with the assistance of strain gauges (Statham Laboratories) and cardiotachometers A complete maternal and a bipolar fetal electrocardiogram were monitored on an oscilloscope and recorded in detail on the polygraph tracing. The maternal body temperature was maintained at 38°C by warm water mattresses

Arterial blood samples were withdrawn from the mother and the fetus at 5- to 15-minute intervals during the experiment. All samples were analyzed for pH and pO_2 using direct reading electrodes (Astrup System Radiometer Copenhagen) The arterial pCO_2 and base excess were derived from the Siggaard-Anderson nomogram (22) In deriving these latter values a similar behavior of monkey and human blood is assumed. In eight experiments the maternal and fetal arterial oxyhemoglobin saturations (O_2Hb) carboxyhemoglobin saturations (COHb) and hemoglobin concentrations (Hb) were measured using a spectrophotometer calibrated for use with rhesus blood (CO-Oximeter Instrumentation Laboratories Inc.). After the withdrawal of each fetal blood sample the fetus was transfused with an equal volume of maternal blood

Following hysterotomy and placement of fetal catheters and electrodes each experiment was initiated with a 20- to 40-minute control period during which the maternal and fetal vital signs and electrocardiograms were observed and frequent measurements of maternal and fetal oxygenation and acid–base status were performed The animals reported here exhibited stable values in the normal range throughout the control period Mean control values were computed for each animal and are depicted as the values at "time zero" in the several figures of the text (In two additional animals, hysterotomy and fetal exteriorization were complicated by fetal hypoxia and acidosis these animals were not subjected to a carbon monoxide insult and are not included in this series)

Following the control period the mother's airway was connected to a reservoir bag into which a carbon monoxide-air mixture was delivered Rebreathing of the mixture was prevented by a Sierra valve The carbon monoxide content of the inspired mixture was regulated between 0.1–0.3 % by employing controlled amounts of two precalibrated CO mixtures. A 0.3 % CO mixture was generally employed initially to produce a rapidly rising maternal COHb level. Subsequent adjustments of the CO concentration were then made according to the severity of the fetal hypoxia observed and the degree of fetal injury intended

The goal of each experiment was to produce either a "moderate" or a "severe" degree of fetal asphyxia as defined below Four fetuses received a "moderate" insult. Four other animals were subjected to a well-documented "severe" insult and a fifth animal in which fetal O_2Hb was not recorded nonetheless had arterial pO_2 values compatible with an insult of "severe" degree. A "moderate" asphyxia was defined as one in which the fetal O_2Hb was held between 12–15% saturation for a period of one hour To produce a "severe" asphyxia the fetal O_2Hb was maintained in the range of 8–11% saturation also for one hour In either instance frequent measurements of the fetal O_2Hb provided feedback data during the experiments which allowed the inspired CO mixture to be so adjusted as to maintain the fetal O_2Hb within the desired range. Occasionally a severely hypoxic fetus exhibited a rapidly worsening bradycardia. Since such an event has been shown to be indicative of a critically worsening fetal asphyxia (17) such episodes were immediately treated by reducing the inspired CO concentration and, when the fetal bradycardia and hypotension failed to respond, by placing the mother briefly on oxygen-enriched air

At the termination of the CO insult, the mother was placed on room air or oxygen by face mask, and the fetus was immediately delivered by surgical section. Every infant was intubated and, when necessary artificially ventilated with 100 % oxygen using an Amsterdam infant respirator (G L. Loos and Co Amsterdam). The degree of oxygen enrichment of the inspired air was rapidly reduced as the animal tolerated it. In a few instances, some oxygen enrichment was required but in reduced amounts over several days. Respiratory assistance when it was required, was withdrawn as the babies were capable of spontaneous breathing

The partial pressures of the respiratory blood gases were determined frequently in the period immediately following delivery in order to document the adequacy of fetal oxygenation and to follow the resolution of any acid–base derangements which might have been incurred during the CO insult. All newborn monkeys were given intensive nursing care in order to optimize their chances of survival The severely injured neonates, at the first signs of decompensation, were anesthetized with pentobarbital and perfused with physiological saline followed by 10 % formalin The brain and spinal cord in each instance were immediately removed for neuropathological investigation.

Calculations

In describing the hypoxia exhibited by the animals of the present series use was made of the blood oxygen content rather than the measured O_2Hb level since the latter fails to take into account observed differences in hemoglobin content and hence in the oxygen-carrying capacity of the blood Both the maternal and the fetal arterial oxygen contents were calculated from their respective measured O_2Hb and hemoglobin values using the formula (7):

O_2 content (ml/100 ml) = $O_2Hb/100 \times Hb$ (g %)$\times 1.39$.
This calculation neglects the minimal quantities of oxygen held in physical solution in the plasma.

All of the measured fetal COHb values were corrected in two ways. The first correction was applied in the presence of low COHb and high O_2Hb (i e during the control period and over the first 15–30 min of CO exposure) when the spectrophotometer systematically overestimated the COHb values In the mother these overestimations amounted to 1 % COHb and could be ignored. In the fetus the various control values of COHb were plotted against their respective values of O_2Hb The resultant curve yielded a straight line having the equation.

Observed COHb (%) = 0 12 × observed O_2Hb (%) + 0 7

The second correction attempted to take account of the small quantities of CO transferred to the fetus via maternal blood transfusions. A value, C_n which represented the cumulative amount of CO in the fetal blood which could be attributed to the transfusion of maternal blood, was subtracted from each measurement of fetal COHb.

Thus, the overall expression which relates the corrected fetal COHb to the measured fetal COHb is.

Corrected $COHb_F$ = measured $COHb_F$ − 0.12 × O_2Hb_F − C_n.

RESULTS

Maternal oxygenation

Inhalation of 0.1–0.3 % CO led to steady rises in the maternal COHb the higher the inspired CO concentration, the more rapid the rise. Alterations in the inspired CO concentration were associated with rapid changes in the slope of the maternal COHb curve (Fig. 1). The maternal arterial O_2Hb measured during the period prior to CO exposure averaged 95.1 % in the animals breathing room air and 100 % in those receiving oxygen. During the CO insult, the measured O_2Hb corresponded closely to the value (100–COHb). This denoted an essential absence of reduced hemoglobin in the blood stream. Thus, the inverse of the maternal COHb curves appearing in Fig. 1 accurately depict the maternal O_2Hb during the period of insult.

The maternal arterial pO_2 on room air ranged from 69–82 mmHg in the nine animals during the control period. As expected (21), this variable remained within the normal range throughout the CO exposures despite profound falls in the maternal arterial O_2Hb *saturation* and oxygen *content*. As such, the maternal arterial pO_2 could, of course not be employed as an index of maternal oxygenation during CO intoxication.

Fetal oxygenation

The rapid rise in the maternal COHb and the corresponding decline in O_2Hb levels led to profound decreases in the measured fetal arterial O_2Hb and in the computed fetal arterial oxygen contents. This effect was particularly dramatic during the first 15–30 min of the maternal CO inhalation, as may be seen from Fig. 1. This initial rapid decline was followed by a plateau characterized by a greatly diminished fetal blood oxygen content. The height of this plateau was defined to a considerable extent by the concentrations of the CO inspired by the mother. As indicated the oxygenation of the fetus could be improved at any time by reducing the concentration of the maternally inspired CO or by administering oxygen-enriched air (Fig. 1). The initial rate of decline of the fetal arterial O_2Hb was 0.5–1.7 % O_2Hb/min for the fetuses given moderate insults (as explained above), and 1.7–2.8 % O_2Hb/min for those exposed to "severe" insults.

Contrasting with the rapidly *evolving* fetal hypoxia the fetal COHb levels rose only slowly during the initial 15–25 min of insult, a time period characterized by a rapidly rising maternal COHb (Fig. 1). A more rapid rise in the fetal COHb first appeared as the maternal COHb approached 60 % or more of its peak value. Subsequent retardations in the rates of rise of the fetal COHb were in every case secondary to induced decreases in the slopes of the maternal COHb curves which preceded them by 10–25 min.

The control values of the fetal arterial pO_2 as obtained while the mothers were breathing room air averaged 25.3 mmHg (S.D. ±5.2). In the three instances in which the maternal arterial pO_2 on oxygen-enriched air was 159, 220 and 428 mmH, the fetal pO_2 was 23, 31 and 38 mmHg, respectively. The fetal arterial pO_2 declined during the insult in a manner consistent with the fall in fetal O_2Hb and oxygen content (see Fig. 2).

The dramatic early decline in the fetal arterial oxygen content upon exposure of the mother to CO appears related to the increasing degree of maternal hypoxia (decrease in O_2Hb) rather than to the development of fetal carboxyhemoglobinemia. The fetal COHb level remained relatively low throughout the CO exposure and the quantities of reduced Hb available continued to be great. Fetal blood oxygen contents as depressed as 1.5–1.9 ml/100 ml were commonly observed when the maternal O_2Hb had fallen below 55 %. In the late stages of the CO exposures, when fetal COHb s in excess of 18 % coexisted with maternal O_2Hb level

Assuming fetal blood volume of 85 ml/kg (4), an average fetal weight of 450 g, and volume of 0.8 ml/transfusion, and letting $COHb_M$ represent the maternal COHb value at the time of the last fetal transfusion prior to the measurement $COHb_F$ it was necessary to reduce each observed fetal COHb reading, $COHb_F$ by the amount C_n where

C_n = 0.021 $COHb_M$ + 0.979 (C + C + C_{n-1})

The expression 0.979 (C + C + C_{n-1}) represents the cumulative contribution of maternal COHb *still* present in the fetal circulation following fetal sample *n*−1

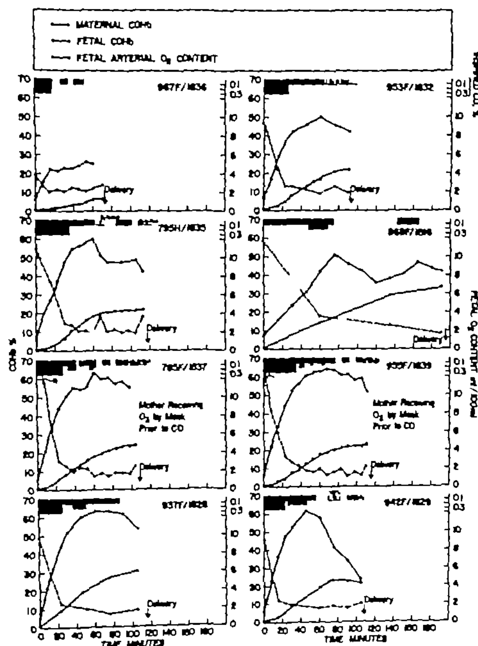


Fig 1 Effects of maternal C[cut off] inhalation fetal COHb and fetal arterial oxygen content The upper 4 [cut off] periments represent 'modera[cut off] the lower 4 severe insults, as defined in the text.

below 55 % the fetal oxygen contents were almost always below 2 0 ml/100 ml

Effects on maternal circulatory and acid base state The control values of the maternal arterial blood pressure averaged 144 mmHg systolic (range = 110–170) and 81 mmHg diastolic (range = 60–110) while the heart rate averaged 185 beats/minute. The CO exposure produced only a slight decline in the maternal blood pressure In 4 animals the systolic blood pressure remained above 125 mmHg throughout while only 2 animals displayed a systolic blood pressure below 100 mmHg and then for a mere 4 and 16 min respectively The CO exposure led to maternal heart rate increases of from 5–50 beats/minute.

In 8 mothers the control pH values ranged fr[cut off] 7 38–7 46 (average 7 42) and the base excess [cut off] in a normal range The ninth animal was sligh[cut off] acidotic exhibiting a control pH of 7.34 and a b[cut off] excess of −6.2 mEq/l The CO exposure p[cut off] duced a slight rise in the arterial pH in all mothe[cut off] reflecting a mild respiratory alkalosis produ[cut off] by an associated hyperpnea The peak mater[cut off] pH attained during the insult averaged 7 47 for [cut off] 9 animals The arterial pCO_2 declined from [cut off] average control value of 35 mmHg (rang[cut off] 31–39) to an average of 26 mmHg (rang[cut off] 21–31) during the insult Aside from the origi[cut off]ly acidotic animal the CO insult produced a fal[cut off] the arterial base excess to below −5.5 mEq/l[cut off]

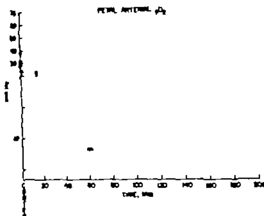


Fig. 2 Individual values of fetal arterial pO_2 as determined during the course of maternal CO exposure. The closed circles represent values obtained from the 4 animals which exhibited no neurological injury the open circles, the data from Fetus 1837 which displayed moderate neurological injury the triangles, the data from the 4 animals exhibiting profound neurological injury (see text)

only 2 animals (in which it reached −6.0 and 8.8 mEq/l).

Two of the adult females showed occasional nodal and/or ventricular premature beats in the electrocardiogram prior to the CO insult. These ECG abnormalities became accentuated during the insult, becoming multifocal in one case. Episodes of bigeminy occurred in both of these animals and these ECG abnormalities were associated with reduced systolic blood pressures. A third animal exhibited short periods of premature ventricular contractions only during the insult. Four animals showed flattening and inversion of the T-wave during the exposure to CO These latter effects were most pronounced in the inferior leads

Effects on fetal circulatory and acid-base state The fetal arterial blood pressure, which averaged 65 mmHg systolic (range = 54–75) and 36 mmHg diastolic (range = 28–40) in the control period, rose by 8–12 mmHg during the first 10–14 min of the CO exposure in 5 animals. The fetal blood pressure then declined steadily in such fashion that it reflected the severity of the fetal hypoxia sustained. Three of the 4 animals undergoing the most severe insults showed systolic blood pressure falling below 40 mmHg for at least 6 minutes during the insult whereas 4 of the 5 animals exposed to a somewhat less severe insult exhibited systolic blood pressures above 45 mmHg at all times.

The fetal heart rate averaged 212 beats/minute in the control period (range = 200–225). Within 15–30 min of the initiation of the CO exposure of the mother the fetal heart rate began to decline steadily to a varying extent in all animals. Three of the 4 fetuses sustaining the most severe insults exhibited a heart rate decline to a level below 100 beats/minute for 10–30 min Fetuses subjected to less severe insults sustained heart rates at levels above 110 beats/minute throughout. The correlation between the fetal heart rate and the fetal arterial blood oxygen content appears in Fig. 3

In 4 experiments mild uterine contractions were present. In these 4 instances, when the fetal heart rate was 125 beats/minute or slower characteristic late decelerations of the fetal heart rate were noted following each uterine contraction. When severe fetal bradycardia was present, administration of oxygen to the mother regularly led to fetal hart rate accelerations of 20–50 beats/minute

The fetal arterial pH which ranged from 7.29 to 7.38 during the control period typically declined

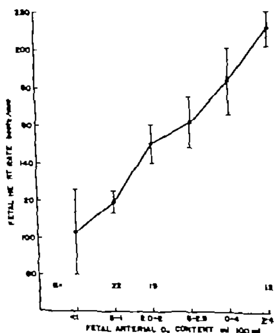


Fig 3 Mean fetal heart rate during maternal CO exposure as function of fetal arterial blood oxygen content. This curve is based upon the pooled data from 9 experiments. The vertical bars represent the 95 % confidence intervals for the mean

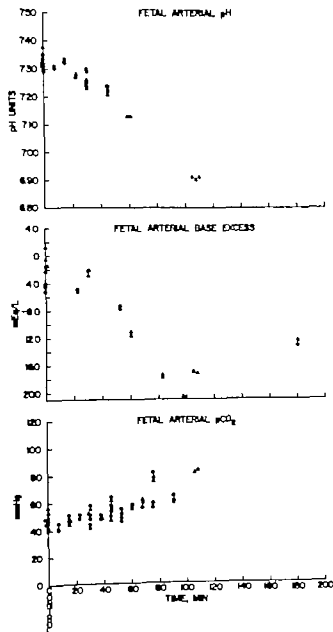


Fig 4 Individual values of fetal arterial pH base excess, and pCO_2 from the 9 experiments during the course of maternal CO exposure Symbols as in Fig. 2

within 30 minutes of the initiation of the maternal CO inhalation Coincident with this decline in pH appeared a depression in the derived arterial base excess (Fig 4) This finding indicated that the fetal acidosis when first apparent was largely of a metabolic type The fetal arterial pCO_2 remained within its control range over the first 60 min of CO exposure. However late in the insult period the pCO_2 tended to rise producing a mixed (respiratory and metabolic) acidosis in the fetus Fig 4 depicts the changes in pH in base excess and in pCO_2 of the fetal arterial blood during the course of maternal CO exposure The degree of fetal hypercarbia tended to correlate with the evolution of arterial hypotension in the fetus as depicted in Fig. 5

An aterial pH below 7 05 and a base excess below −14 mEq/l appeared almost exclusively among those fetuses which subsequently exhibited moderate-to-severe neurological damage and acid-base abnormalities of this magnitude characterized all such fetuses during the second hour of the insult period (Fig. 4)

Following delivery and with the onset of lung breathing the fetal arterial pCO_2 quickly declined toward normal values. The animals subjected to only moderate insults showed restitution of the pH and base excess values to normal within two hours of delivery On the other hand those subjected to severe insults exhibited mild arterial pH depressions (7 23–7.28) for as long as 5 hours following delivery

One-third of the fetuses developed changes in the electrocardiogram during the maternal CO exposure These tended to appear in relation to fetal bradycardia and hypotension and consisted of T wave flattening or inversion

Clinical status and subsequent neuropathology

Following delivery and repair of all incisions the mother in every instance exhibited an uneventful recovery None showed any evidence of neurological impairment during postoperative observation

The clinical and neuropathological findings exhibited by the newborns will be reported in detail elsewhere However in summary the newborns fell into one of three clinical groups Four of the newborns were normal from birth on and remained so until sacrifice several months later Clinical

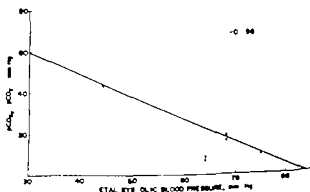


Fig 5 Pooled data from the 9 experiments showing the correlation between the fetal-maternal arterial pCO_2 gradient and the fetal systolic blood pressure.

evaluation of these animals at intervals after birth failed to reveal any definite neurological abnormalities. The brains of these same animals on later pathological study also appeared entirely normal on gross examination. A fifth newborn animal 1037 exhibited moderate neurological impairments when evaluated during the second and third days of life. These findings consisted of hypotonia, lethargy, a poor suck response and occasional brief apneic spells. This animal was sacrificed on the fourth day due to development of gangrene in the left leg and inadequate feeding. Gross examination of the brain in this instance revealed a hemorrhagic necrosis affecting the globus pallidus and putamen bilaterally. Small zones of grossly apparent paracentral cortical necrosis also appeared in both hemispheres.

Four animals were severely damaged. These newborns required prolonged mechanical ventilation because of poor respiratory effort. They showed signs of increased intracranial pressure with splitting of the cranial sutures and prominent retinal hemorrhages. Neurologically they displayed nystagmus, opisthotonus, and intermittent extensor spasms. Death occurred among these animals within 12-72 hours of delivery. Neuropathological examination disclosed widespread hemorrhagic necrosis affecting the cerebral cortex, the basal ganglia, and the thalamus of both hemispheres. These changes were associated with pronounced brain swelling and herniation of the cerebellar tonsils.

Correlations. The degree of neonatal neurological impairment correlated well with the severity of the maternal CO insult and, in particular, with the degree of depression of the fetal arterial blood oxygen content. The 4 surviving and apparently unimpaired newborns had all received insults of "moderate" severity as earlier defined. Their blood oxygen contents during maternal CO exposure had fallen below 2.0 ml/100 ml for at most 10 min and in no case had declined below 1.7 ml/100 ml. In contrast, the 5 newborns exhibiting some degree of manifest neurological damage had all received "severe" insults. *Fetus 1837* which suffered only moderate neurological injury had experienced arterial blood oxygen contents between 1.5 and 2.0 ml/100 ml for 45 min. Three of the 4 severely damaged animals had experienced arterial blood oxygen contents below 2.0 ml/100 ml for 55–73 min during the maternal CO insult. Oxygen contents as low as 1.1 ml/100 ml were observed in this group. The fourth severely injured monkey for which the oxygen contents were not computed, still exhibited pO_2 values during the insult period compatible with this range of oxygen content (Fig. 2).

DISCUSSION

The present study provides evidence that a single acute maternal CO exposure insufficient to produce clinical sequelae in the mother may, nevertheless, lead to dramatic alterations in fetal homeostasis and ultimately severe brain injury or death of the fetus. The data show that a fetal hypoxia of severe degree develops within minutes after the onset of maternal CO exposure in a manner that parallels the CO-induced decline in maternal arterial O_2Hb. This early fetal hypoxia does not result primarily from fetal accumulation of CO inasmuch as the latter occurs quite slowly in the fetus, and, indeed, CO becomes bound to only a small proportion of the total available hemoglobin of the fetal blood. Throughout these episodes, significant amounts of the hemoglobin of the fetal blood remain unsaturated with respect to both oxygen and CO.

In the present study, as in others (16, 17), fetal bradycardia appears as an indicator of fetal hypoxia (Fig. 3). The evolution of a metabolic acidosis and, later, of hypotension in the fetus appears correlated with the severe fetal hypoxia. Cessation of maternal CO administration when coupled with oxygen inhalation by the mother brings about a partial restitution of fetal oxygenation. Rapid surgical delivery and ventilation of the newborn produces a still more dramatic rise in the blood oxygen content and rapidly restores fetal vital functions.

Of particular interest in the present study is the finding that the degree of neurological deficit and brain damage exhibited by the newborns tends to correlate well with the magnitude of the hypoxic insult sustained *in utero*. The data suggest that severe degrees of brain injury first result when a critical level of fetal arterial oxygen content (approximately 2.0 ml/100 ml) is surpassed for prolonged periods. In addition, it is possible that the *fetal acidosis, hypotension* and hypercarbia which appear as concomitants of severe intrauterine hypoxia may themselves contribute to the production of fetal brain damage.

Adequate fetal oxygenation depends upon the proper functioning of the oxygen-carrying mechanisms of the maternal and fetal blood the integrity of the maternal and fetal blood flows through the placenta, and an unimpaired gas diffusion within the placenta itself (12) The present model of CO intoxication exemplifies the malfunctioning of this gas transfer system at several points

As is well known from the early work of Haldane and the later exhaustive studies of Roughton and collaborators (21) the inspired CO not only combines with hemoglobin with an affinity 220–290 times greater than that of oxygen but also causes a leftward shift in the oxygen dissociation curve of the remaining hemoglobin rendering it less capable of discharging its oxygen at a given oxygen tension In addition the oxyhemoglobin dissociation curve of normal fetal blood already lies to the left of the adult curve (12) allowing the fetal blood to discharge its oxygen more effectively at the low oxygen tensions characteristic of fetal tissues In the rhesus this difference between the fetal and the adult oxyhemoglobin dissociation curves is even greater than in the human (18) The early rapid rise in the maternal COHb level which occurs in the present study thus results in a closer approximation of the fetal and the adult curves as well as in a reduction in the oxygen saturation of maternal hemoglobin The actions of these two effects together dramatically impair the net oxygen transfer at the placenta. As the mother continues to inhale CO the slow accumulation of COHb in the fetal blood leads eventually to a leftward shift of the fetal oxyhemoglobin dissociation curve as well (11) This latter effect should bring about a further reduction in the net oxygen delivery to the fetal tissues

Under normal circumstances carbon dioxide diffuses readily across the placental membrane from the fetal to the maternal circulations This transfer is assisted by the fall in maternal and the rise in fetal pCO_2 which occur as the maternal hemoglobin releases its oxygen to fetal hemoglobin (the double Haldane effect ') (20) Such a fetal–maternal pCO_2 gradient exists in the human (23) and in the experimental animal (2) and tends to remain constant despite sizable variations in the maternal pCO_2 (?) A non-uniform distribution of maternal-to-fetal blood flow in the placenta appears to account for the greater part of this gradient (10 12) Maternal hypoxia of short duration has been reported to lead to a more uniform distribution of the fetal and maternal blood flows within the placenta in studies employing radioactive macroaggregates of albumin in sheep (19) In the present study however a significant fetal hypercarbia developed despite the presence of a normal or a reduced maternal pCO_2 (Fig 4) This widening of the fetal-maternal pCO_2 gradient appears to relate in part to the development of significant arterial hypotension in the fetus (Fig 5). These data thus suggest that with the development of severe hypoxia a significant impediment to net placental gas transfer arises due either to a diminished placental perfusion by a hypotensive fetus to an altered distribution of placental blood flows or to an impaired maternal placental perfusion

The slight fetal hypertension which appeared early during the CO insult has been observed by others and has been interpreted as a compensatory response to an impaired oxygen delivery to the fetus (12) In the present study however this hypertension was short lived giving way to a progressive fetal hypotension which in turn was ultimately associated with an impaired placental gas exchange In addition the accumulation of fixed acids and later of carbon dioxide by the fetus would tend to reduce the Bohr effect by which the normally occurring rise in pH in the fetal blood during placental passage increases the affinity of the fetal hemoglobin for oxygen (?0)

The slowness with which carbon monoxide accumulates in the fetal bloodstream is apparent from the present study and has also been noted by others (5 11) Recent studies on the kinetics of CO diffusion across the sheep placenta (9) have shown that the introduction of low concentrations of CO into a closed rebreathing system produces a rapid rise and a subsequent slow decline in the maternal COHb and a slow rise of fetal COHb The rate of accumulation of CO by the fetus is proportional to the difference of the mean partial pressures of CO in the maternal and the fetal blood suggesting that the placental transfer of CO is limited by rates of diffusion rather than by the maternal or fetal blood flows (9)

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The contributions of Messrs. William Rodriguez and E teban Monell Torrens in assisting in the surgical preparations and the analysis of blood samples are gratefully acknowledged

REFERENCES

1. Beaudoing, A. Gachon, J. Butin, L.-P. et al., Les conséquences foetales de l'intoxication oxycarbonée de la mère. Pédiatrie 24: 539, 1969
2. Blechner, J. N., Meschia, G. & Barron, D. H., A study of the acid-base balance of fetal sheep and goats. Quart J Exper Physiol 45: 60, 1960.
3. Brokes, F. Intoxication zweier Schwangeren mit Holzkohlengas. Tod und vorzeitige Geburt eines Kindes. Mschr Geburtsk Frauenkr 13: 449, 1859
4. Brines, J. K., Gibson II, J. G. & Kunkel, P. The blood volume in normal infants and children. J Pediat 18: 447, 1941
5. Curtis, G. W., Algeri, E. J., McBay, A. J. & Ford, R. The transplacental diffusion of carbon monoxide. Arch Pathol 59: 677, 1955.
6. DiGiacomo, R. F. & Shaughnessy, P. W. Estimation of gestational age and birth weight in the rhesus monkey (Macaca mulatta). Amer J Obstet Gynecol 112: 619, 1972.
7. Eilers, R. J. Notification of final adoption of an international method and standard solution for hemoglobinometry specifications for preparation of standard solution. Amer J Clin Pathol 47: 212, 1967
8. Goldsmith, J. R., Contribution of motor vehicle exhaust, industry and cigarette smoking to community carbon monoxide exposures. Ann NY Acad Sci 174: 122, 1970.
9. Longo, L. D., Power, G. G. & Forster II, R. E., Respiratory function of the placenta as determined with carbon monoxide in sheep and dogs. J Clin Invest 46: 812, 1967
10. Longo, L. D. & Power, G. G., Analyses of pO_2 and pCO_2 differences between maternal and fetal blood in the placenta. J Appl Physiol 26: 48, 1969
11. Longo, L. D. Carbon monoxide in the pregnant mother and fetus and its exchange across the placenta. Ann NY Acad Sci 174: 313, 1970
12. Metcalfe, J., Bartels, H. & Moll, W. Gas exchange in the pregnant uterus. Physiol Rev 47: 782, 1967
13. Muller, G. L. & Graham, S. Intrauterine death of the fetus due to accidental carbon monoxide poisoning. New Engl J Med 252: 1075, 1955.
14. Myers, R. E., Beard, R. & Adamsons, K., Brain swelling in the newborn rhesus monkey following prolonged partial asphyxia. Neurology (Minneap) 19: 1012, 1969
15. Myers, R. E., The pathology of the rhesus monkey placenta. *I*. The Use of Non-Human Primates in Research on Human Reproduction (ed. E. Diczfalusy and C. C. Standley) p. 221. Karolinska Institutet, Stockholm.
16. Myers, R. E., Two patterns of perinatal brain damage and their conditions of occurrence. Amer J Obstet Gynecol 112: 246, 1972.
17. Myers, R. E., Mueller-Heubach, E. & Adamsons, K., Predictability of the state of fetal oxygenation from quantitative analysis of components of late deceleration. Amer J Obstet Gynecol 115: 1083, 1973.
18. Novy, M. J., Parer, J. T. & Behrman, R. E., Equations and nomograms for blood-oxygen dissociation curves in adult and fetal macaques. J Appl Physiol 26: 339, 1969
19. Power, G. G., Longo, L. D., Wagner, H. N., Jr et al., Uneven distribution of maternal and fetal placental blood flow as demonstrated using macroaggregates, and its response to hypoxia. J Clin Invest 46: 2053, 1967
20. Power, G. G. & Longo, L. D. Graphical analysis of maternal and fetal exchange of O_2 and CO_2. J Appl Physiol 26: 38, 1969
21. Roughton, F. J. W. & Darling, R. C. The effect of carbon monoxide on the oxyhemoglobin dissociation curve. Amer J Physiol 141: 17, 1944
22. Siggaard-Andersen, O. The pH-log pCO_2 blood acid-base nomogram revised. Scand J Clin Lab Invest 14: 598, 1962.
23. Stamger, V., Eitzman, D., Gessner, I. et al. A study of the acid-base balance of the fetus and newborn and its relation to that of the mother. Amer J Obstet Gynecol 90: 625, 1964

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THE DHEA-S LOADING TEST IN THE EVALUATION OF FETOPLACENTAL FUNCTION

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Abstract. The DHEA S loading test was used to evaluate fetoplacental function in 105 pregnant women. The test gave 84% correct results in the 43 cases of fetoplacental deficiency. However, in the control group of 62 subjects it gave only 42% correct results. Measurement of estriol excretion in maternal urine is one of the biochemical tests routinely carried out on pregnancies at risk. The prognostic value of the test is, however, restricted by the big daily fluctuations in estriol excretion. Fluctuations of between 60% and 140% have been reported (12). In addition to the routine determination of estriol levels, the following tests are of importance for the biochemical evaluation of fetoplacental function: 15α-hydroxyestriol (7), pregnanediol (5), placental lactogen (8), heat-stable alkaline phosphatase (8, 14), and finally, the test discussed in this publication, the dehydroepiandrosterone sulphate (DHEA-S) loading test (6). The present investigation evaluated the usefulness of this test in the early detection of danger to the fetus.

CLINICAL MATERIAL

The clinical material consisted of 105 patients aged from 17 to 43 years, 70 of them primigravidae and 35 multigravidae. Of these, 43 were treated in hospital on account of toxaemia of pregnancy (pre-eclampsia), hypertension and diabetes mellitus. The control group consisted of 62 subjects with approximately normal course of pregnancy apart from two cases they were in hospital for observation.

The clinical material was divided into four groups. The first group consisted of the cases with babies whose weight at birth lay below the 10-percentile limit in the Standard Weight Graphs of Bäckström & Kauppinen (Fig. 1) (1). This group also contained two cases of intrauterine fetal death. The second group consisted of cases with babies whose weight at birth lay below the 25-percentile limit. The third group consisted of cases with babies of normal weight at birth which showed signs of neonatal asphyxia. Apgar tests carried out on these infants 1 or 5 minutes after birth showed maximum values of 6. The fourth group, the control subjects, were mainly patients admitted to hospital for observation on account of slight edema, suspected irregularities in the fetal heart sounds (normal in the CTG) referred by the antenatal clinic, as well as breech presentations, premature labour contractions, etc. In this group the weight of the babies at birth was *over the 25-percentile limit*. The Apgar values and the fetal heart sounds in the CTG as well as the findings at amnioscopy were normal.

The DHEA S loading test was repeated twice or more in 22 patients. It was carried out 136 times in all.

METHOD

The test was conducted in accordance with Lauritzen's method (11), the estriol excretion in the 24-hour collection of urine was determined according to the method of Brown et al. (2) before and after the intravenous injection of 50 mg dehydroepiandrosterone sulphate. When the estriol excretion level after administration of DHEA-S exceeded the basic level by more than 25%, it was regarded as evidence of normal fetoplacental function. Thus in the first three groups the result of the test was designated as correct if the increase in estriol level was less than the 25% mentioned above, or there was no increase at all. On the other hand, it was designated as false-positive if the estriol level exceeded the basic level by more than 25%. If estriol excretion in the control group increased by more than 25% above the basic level after the administration of DHEA-S, the result was designated as correct. In the absence of such an increase the result was designated as false-negative.

RESULTS

The results of the DHEA S loading test are summarized in Table 1. They were excellent in the first group in which there was a high degree of placental insufficiency: in 13 out of 14 cases the test gave a

We should like to thank F. Hoffmann-La Roche & Co. Ltd for providing us with the preparation.

Table 1 *Results of the dehydroepiandrosterone sulphate (DHEA S) loading test*

	Weight at birth of babies under the 10-percentile limit	Weight at birth of babies under the 25-percentile limit	Asphyxiated babies with normal weight at birth	Total	Control group
No of cases	14	17	12	43	62
DHEA-S test correct	13	12	11	36 (84%)	26 (42%)
DHEA S test false-positive	1	3		4	
DHEA-S test false-negative					31 (50%)
On repetition of test, both correct and false results		2	1	3	5 (8%)

fetal malnutrition the babies are then described as small-for-date or as hypotrophic. Both forms are frequently associated with toxemia of pregnancy hypertension or diabetes but may also occur in prolonged and multiple pregnancies. Neonatal asphyxia occurs in acute placental insufficiency

In the present investigation the reliability of the DHEA S loading test was evaluated in accordance with the Standard Weight Graphs of Bäckström & Kauppinen (Fig. 1) and the Apgar scores of the babies. The test gave the correct result in 84% of the women admitted for treatment. On the other hand only 42% of the results were correct in the control group (62 patients). The results in the first and third groups of patients were excellent Surprisingly the test gave a false-positive result in a case of fetal death This was the only failure of the test in the first group In the asphyxiated group the result was correct in 11 cases out of 12. In one case repetition of the test resulted in both a correct and a false positive finding The estimation of estriol excretion provided correct findings in 50% of patients in the first group compared with only 17% in the third group In the second group where the weight at birth of the babies was under the 25-percentile limit, the result of the DHEA S loading test was correct in 71% of cases In 2 cases repetition of the test resulted in both a correct and false-positive finding. It is presumed that a fraction of this group presented slight impairment of fetoplacental function. As already mentioned, the *DHEA-S loading* test gave only 42% correct results in the control group In 50% of the cases with a weight at birth above the 25-percentile limit and with an Apgar score of more than 6, the test wrongly indicated a dangerous intrauterine complication. In 8% of the control group repetition of the test resulted in both a correct and false-negative finding. These results show that the prognostic reliability of the DHEA S loading test is open to question On the other hand apart from 2 cases, the estriol levels in the second and control groups were within normal limits The greater the degree of impairment of fetoplacental function, the more reliable the DHEA S loading test appears to be The test is unreliable in low grade fetoplacental insufficiency and its inaccuracy in cases with a fairly normal course of pregnancy must be appreciated.

REFERENCES

1 Bäckström, L. & Kauppinen, M. A Lapsen sikiökehityksen painonlaskevan. Suom Lääk 23 1553 1968
2 Brown, J B. McLeod, S C. Macnaughtan, C Smith M. A. & Smith B A rapid method for estimating oestrogens in urine using a semiautomatic extractor J Endocrinol 5 42, 1968
3 Crabben, H. van der Haering, M. & Werner Ch. Die Bedeutung des DHEA-S-Belastungstests für die antenatale Beurteilung des Morbus haemolyticus fetalis Geburtsh Frauenheilk 31 844 1971
4 Crabben H van der Haarmacher K. Werner Ch. & Kaiser E Die Beurteilung der Plazentafunktion durch DHEA S-Belastung im Vergleich zu Kardiotokographie und Plazentahistologie Geburtsh Frauenheilk 30 71 1970.
5 Crabben H. van der Kaiser E & Werner Ch. The dehydroepiandrosterone sulphate placental function test J Perinatal Medicine vol II, p 94 S Karger London (1970) Basle (1971)
6 Gruenwald, P Chronic fetal distress and placental insufficiency Biol Neonat 5 15 1963
7 Heikkilä, J & Luukkainen T Urinary excretion of estriol and 15α-hydroxyestriol in complicated pregnancies Amer J Obstet Gynecol 110 509 1971
8 Keller P J Bader P Schmid, J Baerrische U Gerber C & Soltermann R. Biochemical detection of fetoplacental distress in risk pregnancies Lancet II 729 1971
9 Krantz, K. E. & Kubli, F Die Formen der Plazentainsuffizienz. In Gynäkologie und Geburtshilfe Bd. II p 48 Georg Thieme Stuttgart, 1967

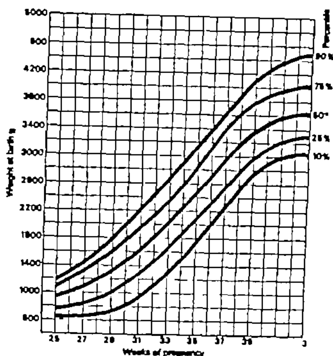


Fig 1 Standard Weight Graphs in accordance with Bäckström & Kauppinen (1)

correct finding In the second group in which there was probably slightly deficient placental function the proportion of correct results was 71% Repetition of the test in two cases gave both correct and falsepositive results In the third group in which the tests were generally carried out 7 days before the birth of an asphyxiated baby the results were once more excellent the finding was correct in 11 cases out of 12 Repetition of the test in this group produced both a correct and a false positive result in one case In the control group on the other hand there were only 42% correct results On repetition of the test the result in 5 cases was both correct and falsenegative.

Pregnancy was terminated by cesarean section in 6 cases with the first group 7 with the second 8 with the third and in 15 with the control group In the first group there were ? cases of fetal death The second and third groups each lost one infant and in the control group the only fetal death was the sec ond of a pair of twins

No side effects arising from the DHEA S loading test were observed

DISCUSSION

Estriol excretion is considered to be a relatively sensitive indicator of fetal and placental disorders (10) The metabolization of DHEA S to estrogens in the fetus and the placenta is dependent on the oxygen supply Hence a decrease in placental circulation may cause a reduction in fetoplacental hormone production

The dehydroepiandrosterone sulphate loading test was first suggested by Lauritzen (11) who considered that the placenta was responsible for the transformation of dehydroepiandrosterone sulphate into estrogens He found a marked difference in the metabolic rates of DHEA-S in normal and pathological pregnancies The results of the DHEA-S loading test were investigated clinically by van der Crabben et al (4) and by Keller et al (8) In order to save time van der Crabben et al modified the test in such a way that it was possible to pronounce an opinion about fetoplacental function within 12 hours (3 5) In order to estimate estriol excretion a 2-hour collection of urine was made. Then 30 mg DHEA-S was injected intravenously and estriol excretion measured in ?-hourly collections. In normal pregnancy the metabolism of DHEA-S to estriol was maximal during the first 2 hours after the injection compared with after 4–6 hours in insufficient fetoplacental function

Lauritzen & Lehmann (1?) administered labelled DHEA S intravenously to the mother They established that within a few minutes approximately 30% of the steroid crossed the placenta from mother to fetus It was rapidly metabolized to estrone both on the fetal and maternal sides Of the transformed end-products of the DHEA S injected 9–16% was rapidly excreted in the urine estriol being quantitatively the most important estrogen in the maternal urine the estriol excreted was in fact 9–13% of the total labelled DHEA S administered In complicated pregnancies the concentrations of labelled DHEA S and estrogens were considerably reduced on the fetal side

The DHEA S loading test carried out by the method of investigation employed by the authors and mentioned above has the same disadvantages as the estimation of estriol excretion in the ?4-hour collection of urine the result is often relatively late in arriving forther renal function may be depressed in toxemia of pregnancy

Placental dysfunction is responsible for 25–40% of all perinatal deaths (9) Gruenwald (6) differentiates between chronic subacute and acute placental insufficiencies with fluctuating boundaries between the individual forms

Both the chronic and the subacute forms cause

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SCREENED GLUCOSURIA DURING PREGNANCY

Correlation with Intravenous Glucose Tolerance Test and Serum Lipids

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Abstract 216 pregnant women with morning glucosuria observed twice at regular testing were subjected to intravenous glucose tolerance tests and serum lipid determinations. A Control Series consisted of 44 pregnant women. An additional group of 24 women with special obstetric histories was also studied. K-values less than 1.0, pointing to diabetes mellitus, were observed in 4.4% of pregnant women with glucosuria, in 3.5% of the control group and in 26.1% of women with special obstetric histories. No positive correlations were found between the K-value and the duration of pregnancy at the time this glucosuria was detected, number of previous pregnancies, weight of newborn, maternal glucose content and 60 min glucose value during IVGTT. The serum lipid values (triglycerides, total and free cholesterol and non-esterified fatty acids) were clearly elevated in all the groups. No correlation was found between the K-value and serum lipid levels. It is concluded that the significance of screened glucosuria, even if recorded twice during pregnancy is questionable. It is pointed out that it is worthwhile to study other groups, such as pregnant women with special obstetric histories because of the high incidence of diabetes mellitus

Glucosuria during pregnancy is a finding which implies the need for further examination of the woman with respect to carbohydrate metabolism. It is well known that apart from manifest diabetes mellitus latent diabetes mellitus is also potentially dangerous t the foetus The risks can be reduced by efficient control of carbohydrate metabolism during pregnancy In practice all patients with glucosuria during pregnancy should be submitted to glucose tolerance test. However this constitutes a considerable load for the maternity and laboratory services It would thus be of value if the significance of glucosuria during pregnancy could be evaluated using another simple screening test.

The aim of our study was to examine the significance of glucosuria recorded twice during pregnancy in connection with regular antenatal check ups, using an intravenous glucose tolerance test (IVGTT) Furthermore the correlations of fasting serum lipids and IVGTT were examined in order to elucidate whether lipid determinations could be used as additional screening tests when evaluating pregnant women with glucosuria.

MATERIAL AND METHODS

The series consisted of 216 pregnant women who at routine antenatal checkups had been found at least twice to have glucosuria. Morning urine glucose was analysed qualitatively using the Clinistix method. If glucosuria was found, an IVGTT was performed in the outpatient department of the university hospital. At the same time the fasting serum lipids were determined

The control series consisted of 44 pregnant women who had been admitted for further examinations because of suspicion of anemia None of these control patients had glucosuria during pregnancy As an additional material, 24 pregnant women presenting with special obstetric histories (babies over 4500 g, malformation of the foetus intra-uterine death, etc during earlier pregnancies) were examined. This group consisted of slightly older women than the two other groups which were remarkably similar in this respect The height, weight and number of earlier pregnancies were similar in all the groups.

IVGTT was performed using 0.5 g glucose per kg of body weight and blood samples for blood sugar deter

10. Kubli F Die chronische Placentarinsuffizienz. Der Gynäkologe I 53 1968
11 Lauritzen C Ausscheidung und Stoffwechsel von Dehydroepiandrosteron in der normalen und pathologischen Schwangerschaft 13 Symposium der Deutschen Gesellschaft für Endokrinologie p 313 1967
12 Lauritzen Ch. & Lehmann W D. Untersuchungen über die physiopathologischen Grundlagen des Dehydroepiandrosteron-Belastungstests bei normaler und bedrohter Schwangerschaft 39 Tagung der Deutschen Gesellschaft für Gynäkologie p 42, 1972.
13 Macnaughton, M C. Hormone excretion as a measurement of fetal growth and development. Amer J Obstet Gynecol 97 998, 1967
14 Tatra, G Caucig, H & Seidl A Hitzestabile alkalische Phosphatase bei normaler und pathologischer Schwangerschaft Geburtsh Frauenheilk 3? 3? 197?

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MATERIAL AND METHODS

The series consisted of 216 pregnant women who at routine antenatal checkups had been found at least twice to have glucosuria. Morning urine glucose was analysed qualitatively using the Clinistix method. If glucosuria was found, an IVGTT was performed in the outpatient department of the university hospital. At the same time the fasting serum lipids were determined.

The control series consisted of 44 pregnant women who had been admitted for further examinations because of suspicion of anemia. None of these control patients had glucosuria during pregnancy. As an additional material, 24 pregnant women presenting with special obstetric histories (babies over 4500 g, malformations of the foetus, intra-uterine death etc. during earlier pregnancies) were examined. This group consisted of slightly older women than the two other groups, which were remarkably similar in this respect. The height, weight and number of earlier pregnancies were similar in all the groups.

IVGTT was performed using 0.5 g glucose per kg of body weight and blood samples for blood sugar deter

Table I *The K value distribution in women with glucosuria control patients and women with special obstetric histories*

K	Glucosuria n	%	p	Control n	%	Women with special obstetric histories n	%	p	p
<1 0	8	4 4	<0 05	4	5.5	6	26.1	<0.01	<0 01
1 0–1 19	17	9 4		3	4 1	6	26.1		
1 2–1 79	109	60 2		45	61 6	9	39 1		
1.8–	47	26 0		21	28 8	2	8.7		
	181	100		73	100	23	100		

p_1 = Significance between glucosuria and the control groups
p_2 = Significance between the control group and the group including the women with special obstetric histories.

Table II *K values (mean ±S D) in different age groups*

Age	Glucosuria n	K	Women with special obstetric histories n	K
<20	43	1 65±0 37	–	–
1–30	103	1 60±0 41	11	1 39±0.48
31–40	32	1.57±0.33	10	1 08±0 16
>40	2	1.51±0 20	–	–
	180		21	

minations were drawn at 10 min intervals during one hour after glucose injection The K value reflecting the disappearance rate of glucose from the circulation was determined according to Lundbæk (6) K-values above 1 20 are regarded as normal K values between 1 00 and 1 19 as borderline and values less than 1 00 as diabetic Blood sugar was determined from capillary blood samples using the enzymatic method of Hyvärinen & Nikkilä (3).

Serum triglycerides, total and free cholesterol and non-esterified fatty acids were determined from fasting blood samples according to a method described earlier by Kallio (4).

RESULTS

The K value distribution in glucosuria and control groups is presented in Table I It can be seen that the K value was less than 1 2 in 50% of women with special obstetric histories There was also a statistically significant difference between glucosuria and control groups in the number of patients presenting with K value less than 1 2 However no difference was found in the number of cases presenting with clearly diabetic K value in these two groups

The effect of age on K value is presented in Table II No difference in this respect was observed between the glucosuria and control groups In women with a special obstetric history it was observed that the glucose tolerance was reduced in the age group between 31 and 40 years

The duration of pregnancy at the time when glucosuria was observed bears no relation to the K value (Table III) Neither was there any correlation between the number of previous pregnancies and the K value (Table IV) In women with special obstetric histories the K values were significantly smaller than the corresponding values in the con-

Table III *K values (mean ±S.D) correlated to duration of pregnancy*

Duration of pregnancy (weeks)	Glucosuria n	K	Control n	K	Women with special obstetric histories n	K
20	2	1.52±0.35	3	1 95±0.79	–	–
21–30	41	1.66±0.40	15	1 64±0 33	1	1 ?4±0.00
31–40	122	1 60±0.37	46	1 60±0 38	3	1 03±0.07
Post partum	10	1 60±0.50	7	1 90±0 64	19	1 25±0.42
	175		71		23	

Table IV. K-values (mean ±S.D.) correlated to the number of previous pregnancies

Number of earlier pregnancies	Glucosuria		Control		Women with special obstetric histories	
	n	K	n	K	n	K
0	108	1.61±0.40	40	1.59±0.36	6	1.28±0.44
1–2	61	1.60±0.34	27	1.66±0.53	11	1.29±0.41
3–	11	1.63±0.43	5	1.80±0.39	4	1.05±0.16
	180		72		21	

trol group in the case of two or more previous pregnancies.

The weights of placentae and distributions according to K-values are seen in Table V. No significant differences were found between the various groups. Neither were there any *differences in* the mean weights of newborns between the groups nor significant correlations between the K-values and the weight of the newborns (Table VI).

No significant differences were found between the control group and glucosuria group in the maximal glucose content observed during the IVGTT (Table VII). Women with special obstetric histories did not differ significantly from the two other groups. The same was true as regards the 60-minute glucose value.

The results of lipid determinations are presented in Tables VIII to XI.

In Table VIII, serum triglycerides in different groups are divided according to K-value. Triglycerides are clearly elevated in all the groups, the highest values being observed *in women* with special obstetric histories. Women with glucosuria and the control group have almost identical triglyceride values. No clear correlation was observed between the K-value and triglyceride levels in any of the groups.

Non-esterified fatty acids are presented in Table XI. A clear elevation of these values is observed in all the groups but without correlation to the K-value.

DISCUSSION

Glucosuria during pregnancy is a common finding occurring in approximately 10% of pregnant women (8). When these women have been subjected to glucose tolerance tests, a diabetic response curve has been found in 3 to 15% of cases (2, 8).

Most of these studies are based on peroral glucose tolerance tests which, however, have been criticized as being too sensitive, because of altered resorption due to pregnancy(7). By means of intravenous glucose tolerance tests, the prevalence of diabetes *mellitus in pregnant* women has been found to be 10% (8). This study was based on a series which was selected by including only women who after a controlled fasting period still had glucose in the urine.

It has been suggested that a positive IVGTT is more significant regarding development of overt

Table V. K-values correlated to the weight of placentae (mean±S.D.)

K	Glucosuria	Control	Women with special obstetric histories	Total material
1.2	596±91 n=3	667±118 n=6	683±157 n=12	634±125 n=41
1.21–1.60	598±127 n=47	620±121 n=25	825±143 n=9	628±145 n=86
1.61	586±177 n=75	627±140 n=28	830±20 n=	602±134 n=103

Table VI. K-values correlated to the weight of newborn (mean±S.D.)

K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1.20	3 595±335 n=24	3 743±331 n=6	4 244±687 n=12	3 800±540 n=42
1.21–1.60	3 403±595 n=52	3 624±612 n=25	4 534±893 n=9	3 583±723 n=86
≥1.61	3 408±413 n=76	3 539±425 n=28	4 725±125 n=2	3 470±448 n=106

Table VII *The maximal and 60 min glucose values (mg/100 ml) in different groups (mean ± S D)*

	Glucosuria	Control	Women with special obstetric histories
Maximal value	215.5±28 1 n=177	219 4±31 2 n=71	206 1±33 1 n=23
60 min value	98 6±17 7 n=177	98.5±19 8 n=71	113 9±20.1 n=23

Table VIII *K values and serum triglycerides in different groups (mEg/l mean ± S D)*

K	Glucosuria	Control	Women with special obstetric histories
≤1 20	7.5±2.1 n=7	7 96±2.25 n=6	10.8±2 7 n=7
1 21–1 60	7 96±2.5 n=44	8.51±2.36 n=25	8.3±3 1 n=5
≥1 61	7 93±2.96 n=48	7 2±1 95 n=20	4.8±0 0 n=1

diabetes mellitus than is an abnormal peroral glucose tolerance test (1) In our study there was a significant difference in K value distribution between glucosuria and control groups the former group having more women with K values less than 1 20 However the numbers of patients with a *diabetic response curve were similar in both* groups showing that glucosuria cannot be used as a reliable screening method for diabetes mellitus during pregnancy This conclusion is also supported by the fact that in the group of women

Table IX *K values and free serum cholesterol in different groups (mg/100 ml mean ± S D)*

K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1.20	102.4±20.6 n=7	102.3±9 0 n=6	114.8±19 1 n=4	106.9±21 9 =11
1 21 –1 60	103 0±22.6 n=44	98 0±17.5 n=26	101 6±22.7 n=5	101 2± 1 1 =75
≥1.61	100 0±17 2 n=48	101 9±20.6 n=20	70 0±0.0 n=1	101 1±18.5 =69

Table X *K values and serum cholesterol in different groups (mg/100 ml mean ± S D)*

K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1 20	318 6±61.8 n=7	330 6±34 7 n=6	328 0±42 9 n=4	327.2±51.2 n=16
1 21 –1 60	311 1±52.2 n=44	307 6±48.9 n=26	311 ±51 6 n=5	308.5±51 0 n=75
≥1 61	297 7±44 1 n=48	309.5±55.3 n=20	237 0±0.0 n=1	300.2±48.1 n=69

with special obstetric histories but without glucosuria during pregnancy a diabetic response curve was found in about 50 % of the cases

The serum lipid values observed in our study were in most cases clearly elevated This finding corresponds to those reported earlier (5) No significant differences were observed between the groups studied When the women were divided into subgroups according to K value no significant differences were found in lipid values which thus cannot be used as *additional screening* method when examining pregnant women with glucosuria in maternity care offices

REFERENCES

1 Benjamin F & Casper D J Oral versus intravenous glucose tolerance tests during pregnancy Amer J Obstet Gynecol 94 566 1966.
2. Drury M I & Timoney F J Latent diabetes in pregnancy J Obstet Gynaec Br Comm 77 24 1970.
3 Hyvärinen A & Nikkilä, E A Specific determination of blood glucose with o-toluidine Clin Chim Acta 7 140 196_

Table XI *K values and non-esterified fatty acids in different groups (nEq/l mean ± S D)*

K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1 20	0 74±0.32 =7	1 17±0.21 =6	0 75±0 06 n=3	0 9±0.3 =16
1 21 1 60	0 87±0.[illegible]3 =38	0.83±0.30 n=[illegible]5	0 97±0.08 n=3	0 8±0. n=67
≥1 61	0 9[illegible]±0.[illegible]6 =44	1 01±0 32 =20	0.89±0.0 n=1	0 9±0.2 n=65

4 Kallio V Fat loading test in males with diabetic heredity As compared to controls, diabetics and coronary patients Acta Med Scand, Suppl. 467 1967
5. Kenttunen, A. & Pyörälä, T Serum lipid pattern in normal pregnancy and pre-eclampsia. J Obstet Gynaecol Br Commw *71* 453 1964.
6 Lundbaek, K. Intravenous glucose tolerance as tool in definition and diagnosis of diabetes mellitus. Br Med J *II*, 1507 1962.
7 Ocampo, P T Coseriu V G & Quilligan, E. F Comparison of standard oral glucose tolerance test and rapid intravenous glucose tolerance test in normal pregnancy Obstet Gynecol *24* 580 1964
8. Sutherland, H W Stowers, J M & McKenzie, C. Simplifying the clinical problem of glucosuria in pregnancy Lancet *I* 1069 1970.

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60 min value	98 6±17 7 n=177	98.5±19.8 n=71	113 9±20 1 n=23

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K	Glucosuria	Control	Women with special obstetric histories
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1 21–1 60	7 96±2.5 n=44	8.51±2.36 n=25	8.3±3 1 n=5
≥1 61	7 93±2.96 n=48	7.2±1 95 n=20	4 8±0.0 n=1

diabetes mellitus than is an abnormal peroral glucose tolerance test (1) In our study there was a significant difference in K value distribution between glucosuria and control groups the former group having more women with K values less than 1 20 However the numbers of patients with a diabetic response curve were similar in both groups showing that glucosuria cannot be used as a reliable screening method for diabetes mellitus during pregnancy This conclusion is also supported by the fact that in the group of women

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K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1.20	102 4±20 6 n=7	102.3±9 0 n=6	114 8±19 1 n=4	106 9±21 9 n=11
1.21 –1.60	103 0±22.6 n=44	98 0±17.5 n=26	101 6±22.7 n=5	101.2±21 1 n=75
≥1.61	100 0±17.2 n=48	101 9±20.6 n=20	70 0±0.0 n=1	101 1±18.5 n=69

Table X *K values and serum cholesterol in different groups (mg/100 ml mean ±S D)*

K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1.20	318 6±61 8 n=7	330 6±34 7 n=6	328 0±42.9 n=4	327.2±51.– n=16
1 21 –1 60	311 1±52.2 n=44	307 6±48 9 n=26	311 2±51 6 n=5	308.5±51 0 n=75
≥1 61	297 7±44 1 n=48	309.5±55 3 n=20	237 0±0.0 n=1	300.2±48.1 n=69

with special obstetric histories but without glucosuria during pregnancy a diabetic response curve was found in about 50% of the cases

The serum lipid values observed in our study were in most cases clearly elevated This finding corresponds to those reported earlier (5) No significant differences were observed between the groups studied When the women were divided into subgroups according to K value no significant differences were found in lipid values which thus cannot be used as additional screening method when examining pregnant women with glucosuria in maternity care offices

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Table XI *K values and non-esterified fatty acids in different groups (nEq/l mean ±S D)*

K	Glucosuria	Control	Women with special obstetric histories	Total material
≤1.20	0 74±0.32 n=7	1 17±0 1 n=6	0 75±0 06 n=3	0 9±0.3 n=16
1 1–1 60	0.87±0.–3 n=38	0.83±0.30 n=25	0.97±0.08 =3	0.8±0. n=67
≥1.61	0 9?±0.26 n=44	1 01±0.3 n=20	0.89±0.0 n=1	0 9±0 n=65

Acta Obstet Gynec Scand 53 329–335 1974

PLASMA PROGESTERONE LEVELS IN NORMAL AND ABNORMAL PREGNANCIES

Bo S Lindberg, Bo A Nilsson and Elof D B Johansson

From the Primate Laboratory and the Department of Obstetrics and Gynecology (Head Professor C. Gemzell), University Hospital, Uppsala Sweden

Abstract Plasma progesterone levels were estimated by competitive protein binding in 815 samples from healthy pregnant women with uncomplicated pregnancies. This series includes 32 patients who were followed serially throughout pregnancy. The mean level increased from 47 ng/ml in week 22 to 148 ng/ml in week 41. The spread was large. Individual patients showed very large variations between two consecutive weeks.

Diurnal variations were examined in 7 patients and short-time variations during one hour in 5 patients. Large but non-systematic variations were found in most cases. The maximal difference between values observed over a 24-hour-period was 123 ng/ml and during one hour 130 ng/ml.

Plasma progesterone levels were studied in 87 cases of toxemia of pregnancy, 6 cases of hypertension, 34 cases of Rh-immunization, 37 cases of diabetes and 5 cases of fetal growth retardation of unknown origin. The results indicate that no constant changes occur in plasma progesterone levels in these groups or in cases of impending fetal death.

As the normal limits are very wide, the intraindividual variations large and the progesterone values in high risk pregnancies are inconclusive, plasma progesterone estimates during the latter part of pregnancy seem to be of limited value.

During human pregnancy large amounts of progesterone are produced by the placenta (1, 14). The production rate during the third trimester lies between 200 and 300 mg/day (9). Part of the progesterone produced is metabolized to pregnanediol and excreted in the urine as the 3-glucuronidate (16). The percentage of conversion to pregnanediol seems to vary with the stage of gestation and is influenced by pathological alterations in risk pregnancies (2, 5).

Large day to day variations in the urinary pregnanediol levels have been found. It is thus hardly surprising that the clinical value of serial determinations of urinary pregnanediol in late pregnancy has been limited.

Recently useful methods for the assay of progesterone in plasma have been developed and applied to physiological and clinical studies. A number of reports dealing with the prognostic value of progesterone determinations in complicated pregnancies have been published (8, 12, 17). The number of cases investigated is, however, small and the results are, in many respects, inconclusive. The aim of the present investigation was to determine the normal limits during the latter half of uncomplicated pregnancies, circadian and short-time variations, and to evaluate the prognostic value of progesterone determinations in plasma in high risk pregnancies.

METHOD OF ASSAY

Plasma progesterone was assayed by competitive protein binding (7). Twenty-five or fifty microliters of plasma were extracted. All samples in the present investigation were assayed in duplicate.

Precision

The coefficients of variation were calculated from duplicate determinations of all the samples. The results are given in Table I.

SUBJECTS AND SAMPLINGS

Plasma progesterone levels in normal pregnancies

Only apparently healthy women with uncomplicated pregnancies, regular menstrual cycles and known dates for the last menstrual period were examined. They subsequently gave birth to healthy single in-

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Table I *Precision of assay at different ranges of measurement*

Progesterone level (ng/ml)	Coefficient of variation (%)	No of duplicate determinations
0–50 0	8.3	63
50 1–100 0	17 4	168
100 1–150 0	15.3	165
150 1–200 0	20.2	171
>200 1	22 1	109

fants weighing more than ? 500 g A large number of patients contributed only one sample a few two to three samples while 32 women were followed by serial sampling from the 22nd week to delivery The time schedule and the number of patients from each week are given in Table II

Circadian and short time variations in plasma progesterone

In seven patients in the last month of pregnancy plasma samples were obtained every second or fourth hour over a ?4-hour period In five patients sampling was performed every fifth minute for one hour All samples from the same patient were analysed within the same assay

Plasma progesterone levels in high risk pregnancies

The majority of the patients in this group were treated and delivered at the University Hospital

Table II *Progesterone values during normal pregnancy*

Pregnancy week, number of cases in each week, mean values in ng/ml are given The normal limits (±2 S D) were calculated from the transformed variable $y = \sqrt{x}$ where x is the observed value

Weeks of pregnancy	N	Mean (ng/ml)	Upper limit	Lower limit
22	60	47	90	14
24	62	56	109	18
26	64	52	104	15
28	60	70	130	25
30	63	75	129	33
32	63	83	145	35
34	65	100	177	41
36	66	112	191	50
37	63	117	?05	49
38	63	133	256	43
39	62	128	?39	45
40	64	141	261	51
41	41	148	264	59
42	19	133	221	63

Table III *Patient material Diagnosis classification of severity and number of cases*

Diagnosis	No. of cases	
Pre-eclampsia, mild	73	87
Pre-eclampsia severe	14	
Hypertensive vascular disease without superimposed pre-eclampsia	6	
Rh-immunization, cord blood Hb>8 g/100 ml		54
Rh-immunization, cord blood Hb<8 g/100 ml		
Rh-immunization, stillbirth	3	
Diabetes mellitus White group B+C	28	37
Diabetes mellitus White group D F+R	9	
Fetal growth retardation of unknown origin	5	

of Uppsala. A few patients were treated at the Departments of Obstetrics and Gynecology of four other large hospitals The progesterone assays were performed at the same laboratory

The various *groups of patients* studied and the number of cases in each group are given in Table III

The classification of *toxaemia of pregnancy* was made according to the recommendations of the U S Committee on Maternal Welfare issued in 1952 (13)

All *Rh-immunized* mothers had anti-D-antibodies which were demonstrated with indirect Coombs technique The live born infants were Rh-positive and had a positive direct Coombs test. The stillborn infants showed signs of severe haemolytic disease at autopsy

The White classification was used for the *diabetic* mothers

Retarded fetal growth refers to a small group of patients whose uteri were too small for the gestational age without any obvious explanation other than placental insufficiency of unknown origin. All these infants were small for dates at birth

Statistical methods

As the distribution of the values displayed a positive skewness normal limits were calculated from the mean ± S D of the transformed variable $y = \sqrt{x}$ where x is the observed value Progesterone mean values in the *different pathological groups* were statistically tested against the means in the corresponding gestational weeks in the normal series by the following procedure In order to test the hypothesis of no differences between groups, analysis of variance was performed If this hypothesis was rejected 95% simultaneous confidence limits for differences between group means were calculated according to the Scheffé method (15) The fluctuations in plasma

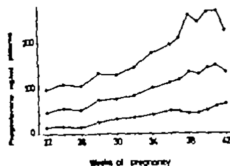


Fig. 1. Plasma progesterone levels in uncomplicated pregnancies from the 22nd to the 42nd week. The number of patients in each week is given in Table II. Mean and variation (±2 S.D.) in ng/ml were calculated from the transformed variable $y = \sqrt{\ }$ where is the observed value.

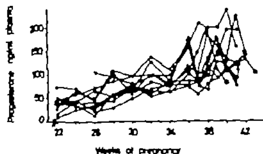


Fig. 2. Individual plasma progesterone levels in 10 randomly selected patients followed from the 22nd week of pregnancy till delivery. Values found at the day of delivery are denoted by circle. The remaining patients followed a similar pattern.

progesterone levels were estimated over 24-hour period and during one hour from analyses of variance (one-way layout) with the model:

$x_{ij} = m_i + e$

x_{ij} = observed value from analyses number j of sample i,
m_i = true mean of sample i,
e_{ij} = error in analyses j of sample i,
σ = standard deviation of sample means (m_i),
σ_e = standard deviation of errors

Estimates of σ and σ are denoted by s_m and s_e and the hypotheses $\sigma = 0$ is tested by the F-test. In this study each sample was analysed twice (j 1,2).

RESULTS

Plasma progesterone during normal pregnancy

There was a steady increase in the mean plasma progesterone levels from 47 ng/ml in the 22nd eek up to 148 ng/ml in the 41st week of pregnancy (Fig. 1 Table II). In the 42nd week a slight decrease was noted. The progesterone values showed an incremental spread with advancing gestational age. No statistically significant difference could be demonstrated between nulliparous and multiparous women.

The progesterone levels in the 32 individual patients examined throughout pregnancy showed in most cases large weekly variations. Differences between two consecutive weeks f more than 50 ng/ml were observed in .5 out of 3. patients and more than 100 ng/ml in 5 out of 3 patients (Fig. 2).

Circadian and short-tim ariations

Large ariations in plasma progesterone levels were found over a 4-hour-period (Table IV). The maximal difference between values observed during a 24-hour-period was 123 ng/ml. No consistent pattern was however observed in relation to time of the day, meals, sleep or light exercise.

The curves from the short-time studies showed that considerable changes sometimes occurred very rapidly. The maximal difference between values observed during one hour was 150 ng/ml (Table V). The fluctuations were significantly larger than could be explained by the methodological error in three of seven cases in the .4-hour-series and in two of five cases in the short-time-series.

Plasma progesterone I high risk pregnancies
Pre-eclampsia

The plasma progesterone values in mild and severe cases of pre-eclampsia are shown in Fig. 3 and 4

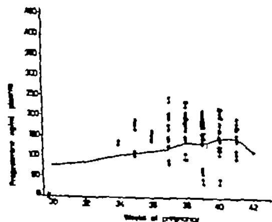


Fig. 3. Plasma progesterone values in 73 cases of mild pre-eclampsia.

Table IV *Variations in plasma progesterone levels during a 24-hour period*

Starting point (hour zero varied from patient to patient All values are given in ng/ml and are means of duplicate determinations. s_m refers to the variance between samples s to the variance within samples. N.s. denotes non significant

Patient	Time of sampling (hours) 0	2	4	6	8	10	12	14	16	18	20	22	24	*P*	s	s_r
VM	182	177	200	220	200	245	209	300	204	180	183	186	207	<0 01	30.6	21.9
IBS	315	233	238	253	238	315	290	267	279	234	-	326	320	<0 05	29.3	31.0
BJ	98	112	100	91	102	97	72	78	65	92	72	90	93	n.s	9.8	13.5
EI	70		86		70		68		84		75		89	n s	3.3	14 4
OP	153		186		163		186		186		136		182	<0.05	17.6	13 4
MS	144		161		132		188		107		148		110	n.s.	21 4	26.5
MB	71		86		70		68		84		75		89	n.s.	7 6	9 4

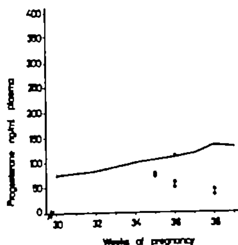


Fig 4 Plasma progesterone values in 14 cases of severe pre-eclampsia.

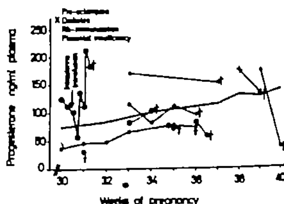


Fig 5 Plasma progesterone values in 10 cases of intrauterine death due to various complications

Both groups showed the same general pattern with most values spread within the normal limits The mean values in both groups were generally some what higher than the corresponding values in the normal series The differences between progesterone levels in pre-eclamptic patients and normal patients were at times significant (weeks 36–38 40–41) at other times not

Intrauterine death occurred in four cases of pre-eclampsia (Fig 5) One showed a sharp drop in plasma progesterone from 174 ng/ml to 38 ng/ml In the other three cases the values were within the normal range and the death of the fetus was not preceded by any recorded dramatic changes in this respect

Table V *Short time variations in plasma progesterone values*
The same symbols as in Table IV are used

Patient	Time of sampling (minutes) 0	5	10	15	20	25	30	35	40	45	50	55	*P*	s	s_r
AL	217	173	287	152	185	190	220	220	177	252	245	282	<0 001	46.2	25.4
SJ	173	195	193	200	178	158	173	185	[illegible]	183	190	158	n s.	12.1	14 3
KE	198	263	200	213	215	173	198	200	220	218	231	303	<0.05	20 0	16 8
ME	91	88	96	104	76	108	110	101	122	95	97	98	n s	7 4	9.5
BL	131	127	146	133	145	143	140	134	177	151	148	166	n.s	8.8	10.2

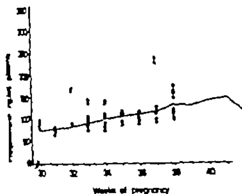


Fig. 6 Plasma progesterone values in Rh-immunized mothers with mild to moderate haemolytic disease (cord blood Hb of more than 8 g/100 ml).

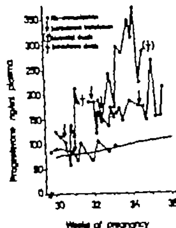


Fig. 8 Plasma progesterone levels in four Rh-immunized patients who received intrauterine blood transfusions.

Hypertensive vascular disease without superimposed pre-eclampsia

In this small group plasma progesterone levels were evenly spread within the normal range. No consistent pattern could be discovered in the distribution of the values.

Rh-immunization

In mild and moderate cases (cord blood Hb more than 8 g/100 ml) the plasma levels showed the same general picture as in patients with pre-eclampsia (Fig. 6). Most values were within the normal range. The mean values for the different weeks were slightly higher than in the normal group. In the severely affected groups most values were located in the upper part of the normal range (Fig. 7). Three infants died in utero (Fig. 5). A dramatic rise in the progesterone levels was observed before the death of the infant in one of the cases. The two other ones showed no dramatic changes. One severely affected infant died neonatally from haemolytic disease. The maternal progesterone level showed a steep rise followed by a drop during the last week before delivery. Two infants survived after one or two intrauterine transfusions (Fig. 8). The progesterone values of one of these mothers fluctuated around the upper normal limit but with one very high value about one week before delivery. In the other cases the values were around the normal mean.

Diabetes mellitus

The plasma progesterone levels of diabetic mothers showed the same general distribution as in pre-eclampsia and Rh-immunization (Fig. 9) with

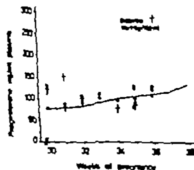


Fig. 7 Plasma progesterone values in Rh-immunized mothers of infants with severe haemolytic disease (cord blood Hb<8 g/100 ml) and in cases with intrauterine fetal death due to severe haemolytic disease.

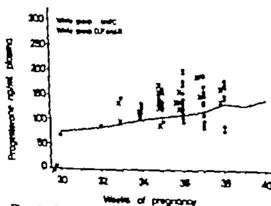


Fig. 9 Plasma progesterone levels in 37 pregnant women with diabetes mellitus.

slightly elevated mean values In one case of intrauterine death a slight fall from 176 ng/ml to 139 ng/ml was noted (Fig. 5)

Fetal growth retardation

In five cases of intrauterine fetal growth retardation with placental insufficiency of unknown origin four had normal plasma progesterone levels One of these infants died in utero In a second case of intra uterine death one value below the lower normal limit was recorded (Fig 5)

DISCUSSION

The levels of plasma progesterone during the second half of the normal pregnancies reported here agree both in pattern and range with previous findings (6 11 19) The gradual increase in progesterone levels may reflect the increase in placental size

The rapid competitive protein binding assay used in this study has a good precision at low levels of progesterone found during the menstrual cycle and early pregnancy (7) At the progesterone levels found at the end of pregnancy the coefficient of variation between duplicate determinations of the same sample is about 20% In spite of this rather large variation our results indicate that there are very large fluctuations in the endogenous progesterone concentrations The fluctuations may occur very rapidly and show no systematic cir cadian rhythm Greig et al (4) when sampling three times a day were unable to find any significant diur nal variation Wiest (18) collected samples six times during a 24-hour-period in seven patients The results of their investigations were not analysed in relation to errors in the measurements but seem to indicate that there are significant but non systematic fluctuations in plasma progesterone levels during a 24-hour-period The large fluctu ations found in the present investigation may be due to the short halflife of progesterone (3) and the rapid conversion and synthesis in placenta

The results from our high risk pregnancies seem to favour the view that changes in the maternal plasma progesterone levels are of little clinical significance No consistent pattern could be dem onstrated in connection with toxaemia of pregnancy Rh-immunization diabetes or retarded fetal growth The mean values in the pathological groups were sometimes significantly higher than the normal means The reason for this may be that in the pathological cases several samples were analysed each week In the statistical calculations only the last sample for each week was used As the progesterone levels showed an increase with advancing gestational age this procedure may lead to slightly higher values than those in the normal group in which only one sample a week was obtained

The large intraindividual variations in progesterone levels and the absence of characteristic progesterone patterns in high risk pregnancies and impending fetal death limit the clinical value of progesterone determinations Human placental lactogen (HPL) has been estimated in the same patient material (10) A comparison between these two hormones of predominantly placental origin suggests that HPL gives more useful information in high risk pregnancies.

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REFERENCES

1 Diczfalusy E. & Troen, P Endocrine functions of the human placenta Vitamines Hormones 19 229 1961
2. Fotherby K Progesterone metabolism in pregnancy Proc Roy Soc Med 63 1061 1970
3 Fylling P Disappearance rate of progesterone following simultaneous removal of the corpus luteum and the foetoplacental unit in women. Acta Endocrinol (Kbh.) 65 284 1970
4 Greig, M Coyle M G Cooper W & Walker J Plasma progesterone in mother and foetus in the second half of human pregnancy J Obstet Gynaec Brit Emp 69 772 1962
5 Henssen, S G F Theses Nijmegen Crouzen Maastricht 1969
6. Johansson E. D B Plasma levels of progesterone in pregnancy measured by a rapid competitive protein binding technique Acta Endocrinol (Kbh) 61 607 1969
7 Johansson E D B Progesterone levels in peripheral plasma during the luteal phase of the normal menstrual cycle measured by a rapid competitive protein binding technique Acta Endocrinol (Kbh) 61 592, 1969
8 Jonasson L. E & Johansson E. D B Progesterone levels in amniotic fluid and plasma from women. Acta Obstet Gynecol Scand 50 345 1971

9 Lin, T J Lin, S. C Erlenmeyer F Kline, I T Underwood, R., Billiar R. B & Little, B., Progesterone production rates during the third trimester of pregnancy in normal women, diabetic women and women with abnormal glucose tolerance. J Clin Endocrinol 34 287 1972.

10. Lindberg, B S & Nilsson B A., To be published.

11. Lurie, M Levanti, S & Fraenchl, F The description and evaluation of competitive protein binding radioassay of plasma progesterone in pregnancy Fertil Steril 22 435 1971

12. Lurie, A O Reid, D D & Villee C. A. The role of the fetus and placenta in maintenance of plasma progesterone. Am J Obstet Gynecol 96 670 1966

13. McCartney C P *I* Obstetrics, 13th ed. (ed. J P Greenhill), p 301 Saunders, Philadelphia and London, 1965

14 Ryan, K. J Hormones of the placenta Am J Obstet Gynecol 84 1695 1962.

15. Scheffé, H., Analyses of variance. Wiley & Sons, New York, 1959

16 Solomon, S. & Fuchs, F *I* Endocrinology of Pregnancy (ed. F Fuchs & A. Klopper), p. 81 Harper & Row New York, Evanston and London, 1971

17 Tulchinsky D Hobel, C. J., Yeager E. & Marshall, J R., Plasma estradiol, estriol and progesterone in human pregnancy Am J Obstet Gynecol *113* 766, 1972.

18 Wiest, W G Estimation of progesterone on biological tissues and fluids from pregnant women by double isotope derivative assay Steroids *10* 279 1967

19 Yannone M. McCrudy J R. & Goldfien, A. Plasma progesterone levels in normal pregnancy labour and the puerperium. Am J Obstet Gynecol *101* 1058, 1968.

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REFERENCES

1 Diczfalusy E & Troen, P Endocrine functions of the human placenta. Vitamines Hormones 19 229 1961
2 Fotherby K. Progesterone metabolism in pregnancy Proc Roy Soc Med 63 1061 1970
3 Fylling, P Disappearance rate of progesterone following simultaneous removal of the corpus luteum and the foetoplacental unit in women. Acta Endocrinol (Kbh) 65 284 1970.
4 Greig, M Coyle M G Cooper W & Walker J Plasma progesterone in mother and foetus in the second half of human pregnancy J Obstet Gynaec Brit Emp 69 772 196_
5 Hermsen S G F Theses, Nijmegen Cronzen, Maastricht, 1969
6. Johansson E. D B Plasma levels of progesterone in pregnancy measured by a rapid competitive protein binding technique Acta Endocrinol (Kbh) 61 607 1969
7 Johansson E D B Progesterone levels in peripheral plasma during the luteal phase of the normal menstrual cycle measured by a rapid competitive protein binding technique Acta Endocrinol (Kbh.) 61 59? 1969
8 Jonasson L. E. & Johansson E D B Progesterone levels in amniotic fluid and plasma from women Acta Obstet Gynecol Scand 50 345 1971

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TOTAL HYSTERECTOMY WITH AN AMPLE VAGINAL CUFF

A combined Vaginal and Abdominal Approach

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Abstract. A combined vaginal and abdominal approach for simple, total hysterectomy including an ample vaginal cuff, is described.

[Re]currence in the vaginal vault occurs in about 2% of cases of preinvasive cancer of the cervix [tre]ated by total hysterectomy (6, 7). The reason for [thi]s recurrence is often that the vaginal cuff at [hys]terectomy has been too narrow.

The importance of an adequate vaginal cuff at [o]peration for preinvasive and invasive cancer of [th]e cervix has been pointed-out by Graham et al., [Gr]ay, Meigs, Scott, and Telinde & Mattingly (2, 3, [4,] 5, 6) among others. Hysterectomy has in these [c]ases been performed abdominally.

Combined abdominal and vaginal procedures [h]ave been used for malignant changes far down in [t]he vagina. Ariel & Trinidad (1) described such a [m]ethod with malignant melanoma in the vagina, [w]here radical abdominal hysterectomy was com[bi]ned with a vaginal dissection of the distal part of the vagina.

Different methods have been recommended for deciding how large a part of the vagina ought to be resected at surgery for cancer of the cervix. Iodine-staining of the upper vagina and biopsies of suspected areas have been utilized by Graham et al., Gray, and Scott (2, 3, 5). Preoperative iodine-staining of the cervix and the upper part of the vagina in combination with colposcopy have been proposed by Telinde & Mattingly (6).

These examinations help establish the level at which the vagina ought to be divided. An incision at a predetermined point on the vaginal wall is, according to Graham et al. ( ), a simple way of marking this level for guidance at abdominal hysterectomy.

The purpose of the present article is to describe a combined vaginal and abdominal approach for simple total hysterectomy which allows removal of an adequate vaginal cuff.

MATERIAL AND METHODS

This technique has been used for operating on 18 patients during the period 1969–1972. Nine patients had an adenocarcinoma of the cervix uteri which was growing superficially. Preoperative radiation therapy was given in the form of external treatment. A dose of 3100±200 rad was given to the true pelvis using two opposing fields with ⁶⁰Co at an SSD of 70 cm, or with 8 MV X-rays from a linear accelerator at an SSD of 100 cm, in 15–17 fractions, one fraction daily 5 days a week. The operations were performed 6 weeks after completion of radiation therapy.

Nine patients had a recurrence after full radiation treatment of squamous carcinoma of the cervix. The recurrent tumour in these cases was judged to be superficial.

Between one-fourth and three-fourths of the vagina was removed surgically. Besides the usual routine examinations, colposcopy was performed prior to the operation. The findings of this examination help decide how much vagina should be removed. The operation is started vaginally with circumcision of the vagina following the subepithelial infiltration of "[illegible]" solution for easier dissection, and for reducing bleeding. The vaginal walls are dissected free and the bladder is separated from the cervix up to the level of the vesico-uterine fold. The rectum is freed from the cervix up to the cul-de-sac. Clamping of the base of the broad ligament and the utero-sacral ligaments is not performed vaginally.

The vaginal cuff is closed with a suture and a pair of chromic catgut sutures are then placed at each corner of the vagina, leaving a gap in the mid-line the width of a finger. A loose pack is inserted up through this gap into the area above the vagina. The pack is removed later when the utero-sacral ligaments and the parametrium are divided.

6. Telinde, R. W. & Mattingly, R. F.: Operative Gynecology. J. B. Lippincott Co., Philadelphia and Toronto, 1970.
7. Topek, N. Y.: Surgical treatment of carcinoma in situ of cervix. Clin Obstet Gynecol *10*: 853, 1967.
8. Richardson, A. C., Lyon, J. B. & Graham, E. E.: Abdominal hysterectomy: Relationship between morbidity and surgical technique. Am J Obstet Gynecol *115*: 953, 1973.

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The procedure is then continued abdominally and is performed as an ordinary hysterectomy except that the broad ligament is opened widely In this way it is usually possible to see the ureters or make them easily palpable without much dissection

When the uterus has been removed the operation is concluded in the usual way In those cases where a large part of the vagina has been removed, some Surgicel® is left above the vagina and a pack is inserted for about 24 hours

Postoperative prophylactic treatment with antibiotics has been given only to patients with recurrent cancer

RESULTS

All of the 18 patients had a satisfactory margin in the vaginal cuff free from the tumour and the operations were carried out without any technical complications Bleeding during the operations has only been slightly greater than at simple abdominal hysterectomy and only one patient was given blood (450 ml) during or after the operation Four patients had temperatures exceeding 38°C for 3 days or longer In one case this was due to infection in the urinary tract One patient had infection in the abdominal wound and one had infection above the vaginal vault The average time in hospital was 10 days (7–17 days) No case of postoperative atony of the bladder was noted

One patient who had recurrent cancer in the cervix spreading superficially to the vagina and who had received full radiation therapy as well as removal of three-fourths of the vagina got a vesico-vaginal fistula 5 months following surgery The other patients have not had any delayed complications in the urinary tract Routine intravenous urography has not however been performed following surgery

DISCUSSION

Removal of an ample vaginal cuff at simple total abdominal hysterectomy can be complicated It can sometimes be difficult to decide where to divide the vagina even at radical hysterectomy The technique described here permits the dissection of a satisfactory vaginal cuff by a vaginal approach Dissection of the distal parts of the ureters is not necessary and disturbances of the blood supply are thereby avoided The dissection of the vaginal cuff is an advantage even in those cases where radical hysterectomy is subsequently found to be necessary

The patient who developed a vesico-vaginal fistula had received full radiation therapy for an invasive cancer of the cervix Radiotherapy causes changes in the vascular bed which result in ischaemia Surgical intervention in such areas may greatly increase the risk of tissue necrosis and this was probably important in the development of a fistula in this case

The postoperative course was uncomplicated in all patients except for those in whom a pyrexia necessitated a longer period of nursing-care than is usual after total hysterectomy (8) As there is little disturbance of the urinary tract it would appear that the frequency of urinary complications can be kept relatively low though this series is too small to permit drawing any decisive conclusions

The operation in this series have been performed for early adenocarcinomas of the cervix which have been given preoperative radiation therapy and for recurrences of squamous carcinoma where the tumour has been judged to be a superficial growth. The technique described here ought to be of use in those cases of preinvasive cervical cancer where hysterectomy is indicated

CONCLUSION

The surgical technique described here can be a useful alternative in those cases where radical hysterectomy is not indicated but where removal of an ample vaginal cuff is necessary

REFERENCES

1 Ariel I M & Trinidad S S Malignant melanoma of the vagina I Treatment of Cancer and Allied Disease vol VI Tumour of the Female Genitalia (ed G T Pack & M A Irving) Harper & Brothers New York 196[illegible]

2 Graham J B Sotto L S J & Paloucek F P Carcinoma of the Cervix W B Saunders Co Philadelphia and London 196[illegible]

3 Gray L A Dysplasia Carcinoma in situ and Micro-Invasive Carcinoma of the Cervix Uteri (Compiled by L A Gray) Thomas Springfield Ill 1964

4 Meigs J V Dysplasia Carcinoma in situ and Micro-Invasive Carcinoma of the Cervix Uteri (Compiled by L A Gray) Thomas Springfield Ill 1964

5 Scott R B Dysplasia Carcinoma in situ and Micro-Invasive Carcinoma of the Cervix Uteri (Compiled by L A Gray) Thomas Springfield Ill 1964

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CYSTINE AMINOPEPTIDASE ACTIVITY IN PREGNANCY

III *A Comparison between Cystine Aminopeptidase Activity in Maternal Blood and Urinary Oestriol Excretion during Pregnancy*

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Abstract. Maternal cystine aminopeptidase activity (CAP) and total urinary oestriol excretion have been compared in 11 normal patients between 30 and 41 weeks gestation, in 9 patients with toxaemia of pregnancy and in 3 patients with history of complication during the actual or previous pregnancy The statistical correlation between maternal CAP and oestriol excretion in normal patients was poor ($r=0.32$). In patients with pre-eclampsia the results were as follows low CAP levels generally were associated with low oestriol excretion, whereas with high or normal CAP values there were varying levels of oestriol CAP levels in blood are only related to the function of the placenta, whereas the oestriol excretion is related to the condition of the whole fetoplacental unit. Therefore serial CAP assays cannot replace oestriol assays in assessing the condition of the foetus and placenta. However in some patients where intrauterine fetal death occurred, decreasing CAP values were observed before there was significant change in oestriol excretion This indicates that decreased placental function might be observed at an earlier stage using CAP than using oestriol determinations CAP assay therefore justified as valuable test alongside estimation of oestriol excretion in judging the condition of the placenta during the last trimester As the methods of CAP assays are simple and reproducible CAP can be used on a large scale for detecting and monitoring pregnancies at risk, in order to get a more diversified picture with respect to the condition of the fetus and placenta during the last trimester

In previous report (9) the results of serial cystine aminopeptidase (CAP) estimations in maternal blood during normal and abnormal pregnancy were presented It was concluded that the value of such estimations as placental function test could not be evaluated until a comparative study with other parameters reflecting the condition of the fetus and the placenta was performed.

Of all the tests available serial urinary oestriol assay is the method most widely used and accepted for this purpose Therefore it seemed reasonable to compare serial CAP assays with oestriol assays in evaluating placental function in normal and abnormal pregnancy

MATERIAL AND METHODS

The patients were divided into the following groups.

Normal patients Criteria of normality were The date of the last menstrual period was certain The uterine size was compatible with the period of amenorrhoea. The blood pressure was below 140/90 and there was no proteinuria. There was no abnormal bleeding during the actual pregnancy

Moderate pre-eclampsia Blood pressure was 140/90 or more but did not exceed 160/100, there was oedema but no proteinuria.

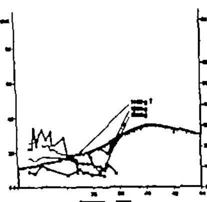
Severe pre-eclampsia Blood pressure was higher than 160/100 and there was oedema and/or proteinuria. Patients with proteinuria but with blood pressure between 140/90 to 160/90 were also classified in this group

Miscellaneous group This group includes one patient with previous history of intra-uterine fetal death and two patients with intra-uterine death in the actual pregnancy

The method of CAP assay and the calculation of the enzyme activity was the same as described previously (10) using cystine-di-β-naphthylamide as substrate

Urinary oestriol excretion was measured using an automated method (5)

Blood samples Maternal blood samples were centrifuged, stored in refrigerator and the estimations usually performed within 48 hours. If the interval before assay exceeded 48 hours the samples were kept at −20°C.



3 Serial CAP assays and oestriol assays in [patients] with severe pre-eclampsia. CAP values within [the] shaded area indicate values beneath the 10th [percentile]. Filled symbols indicate oestriol and un[filled] symbols CAP values. For further explanation [see] text.

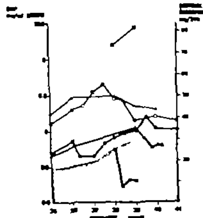


Fig. 4. Serial CAP assays and oestriol assays in 3 patients with severe pre-eclampsia. Filled symbols indicate oestriol and unfilled symbols CAP values. For further explanation see text.

[deli]very was followed with serial CAP (O) and [oes]triol assays (●). On the basis of supposed [pre]maturity induction of labou with oxytocin [wa]s performed but the induction was unsuccess[ful]. Three days later the patient went into [spon]taneous labour. During labour the foetal heart [sou]nds suddenly disappeared. At delivery there [we]re abundant degenerative changes with fibrinoid [in]farcts in the placenta. In this case there was a [pr]onounced (40%) decrease i CAP activity during [th]e last 14 days before labour started whereas [th]e oestriol excretion did not change significantly.

[O]n account of our present knowledge on the slow [eli]mination rate of CAP in blood a decrease of [..]% in CAP level must correspond to drastic [de]crease in placental function and indicate that [del]ivery should be started or continuous fetal [su]pervision be performed.

In another patient (Fig. 5) with twin pregnancy [d]ecreasing CAP levels were observed from the [3]7th week of gestation, whereas the oestriol ex[c]retion was actually increasing. I[n] this patient [th]e heart sounds of one of the twins suddenly [c]eased wherea[s] significant decrease in oestriol [ex]cretion was not obtained until after intra-uterine [d]eath. The weight of the monozygotic placenta [wa]s 910 g and no significant changes were found [i]n the placenta on microscopical examination.

The third patient in this group was [a] diabetic woman with one normal deli[v]ery and thereafter [..] spontaneous [a]bortions. In the actual pregnancy the highest CAP level was found in the 33rd week (Fig. 5) whereas the oestriol values slowly increased to the 36th week. The patient was kept under constant supervision using cardiotocographic recordings. In the 37th week uterine contractions were recorded and at the same time the fetal heart rate decreased to 60–80 beats per minute and became irregular. The patient was delivered by cesarean section (CS) and a dysmature baby weighing 1570 g was born. The immediate development of the baby was uncomplicated.

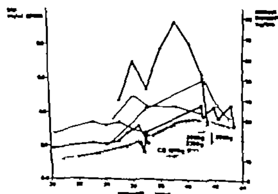


Fig. 5. Serial CAP and oestriol assays in 3 patient with pregnancy complications. ▲, △, twin pregnancy; ■, □, diabetic mellitus and placental insufficiency; ●, O postmaturity. Filled symbols indicate oestriol and unfilled symbols CAP values. The shaded area indicates CAP levels beneath the 10th percentile in normal single pregnancy. For further explanation see text.

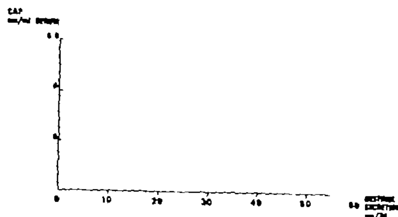


Fig. 1. Individual paired values of serum CAP and urinary oestriol in normal pregnancy between 30 and 41 weeks of pregnancy.

RESULTS

Serial CAP and oestriol assays were performed in 11 normal patients between 30 to 41 weeks of pregnancy totalling 58 estimations. The results are shown in Fig. 1. A regression analysis did not demonstrate any significant correlation between the CAP levels and the oestriol excretion in these patients ($r=0.32$, $P<0.02$).

Serial CAP and oestriol assays were performed in 3 patients with moderate toxaemia of pregnancy (Fig. 2). The delivery was uncomplicated, the birthweight within the normal range and the Apgar score 9 or 10 one minute after delivery. The CAP values were within the normal range in two patients. In one patient the CAP levels were below the 10th percentile from the 37th week until delivery whereas the oestriol values were normal in all cases.

The results obtained in 6 patients with severe toxaemia of pregnancy are summarized in Figs. 3 and 4. Patients with low CAP levels were also found to have low oestriol excretion (Fig. 3). In two cases of intra-uterine fetal death the weight of the babies were 1460 and 1300 g respectively. The third patient, with a pregnancy of 37 weeks, was delivered by cesarean section (CS) due to drop in the oestriol excretion and the low CAP values. The CAP values were in two cases below the normal range and in the third patient (Δ) the CAP values were declining from the 32nd week of pregnancy. The sharp decrease in oestriol excretion (▲) in this latter patient was unfortunately detected the same day as the foetal heart sounds disappeared. In all patients the CAP as well as the oestriol values were low or decreasing. However, the individual variation in CAP assays was generally less pronounced than in the oestriol assays.

The results in 3 patients with severe pre-eclampsia and normal or high CAP values are shown in Fig. 4. One patient (○, ●) was delivered by cesarean section as induction of labour with oxytocin failed. Another patient who came into the hospital with a threatening eclamptic attack had very high CAP levels (□) which in fact increased before delivery, whereas the oestriol values (■) were declining to very low values. In this patient the labour was induced. The weight of the baby was 2650 g but there were pronounced degenerative changes in the placenta. The CAP levels in this patient must have been quite misleading concerning the functional state of the placenta.

The results in three patients with complication during the actual pregnancy are also presented (Fig. 5). One patient with a previous history of oligomenorrhoea and uncertain expected date of

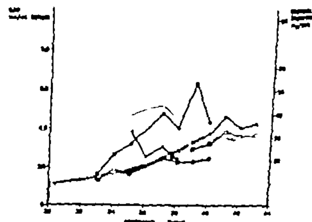


Fig. 2. Serial CAP assays and oestriol assays in 3 patients with moderate toxaemia of pregnancy and normal delivery. Filled symbols indicate oestriol and unfilled symbols CAP values. CAP values within the shaded area indicate values beneath the 10th percentile.

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REFERENCES

1. Bjørø, K., Oestriol assay in obstetric practice. Acta Endocrinol (Kbh) 69 Suppl. 161 1972.
2. Booth, R. T. Stern, M. I. Wood, C., Sharples, M. J. H. & Pinkerton, J. H. M. Urinary oestriol as an index of placental function and foetal viability. J Obstet Gynaecol Br Commw 71 266, 1965
3. Chapman, L. Silk, E., Skupny A., Tooth, E. A. & Barnes, A. Spectrofluorimetric assay of serum cystine aminopeptidase in normal and diabetic pregnancy compared with total oestrogen excretion. J Obstet Gynaecol Br Commw 78, 435 1971
4. Curzen, P. & Varma, R. A comparison of heat stable alkaline phosphatase and urinary oestrogen excretion in the mother as placental function tests. J Obstet Gynaecol Br Commw 78 686, 1971
5. Hainsworth, I. R. & Hall, P. E. A simple automated method for the measurement of oestrogens in the urine of pregnant women. Clin Chem Acta 35 201 1971.
6. Petrucco, O. M., Cellier K. & Fishtall, A.. Diagnosis of intrauterine fetal growth retardation by serial serum oxytocinase urinary oestrogen and serum heat stable alkaline phosphatase (HSAP) estimations in uncomplicated and hypertensive pregnancies. J Obstet Gynecol Br Commw 80 499 1973.
7. Tovey J. E., Sykes, J. R. Robinson, D. A., Hurry D. J. Cooper K. & Beynon, C. L.. Comparison of total urinary estrogens and serum cystine aminopeptidase estimations in pregnancy. Clin Biochem 5 104 1972.
8. Rydén, G., Cystine aminopeptidase activity in pregnancy. A rapid method for estimations of placental function in clinical practice. Acta Obstet Gynecol Scand 50 253 1971
9. – Cystine aminopeptidase activity in pregnancy. Its clinical application as an index of placental function. Acta Obstet Gynecol Scand 51 329 1972.

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DISCUSSION AND CONCLUSION

For many years the estimation of urinary oestriol excretion during the last trimester has been widely accepted as the method of choice to assess function of the feto-placental unit. However the method has some disadvantages mainly the inconvenience and the inaccuracy of the 24-hour urine sampling Even with careful urine collection the individual day-to-day variation of oestriol excretion is rather pronounced (7) and recent reports (1 4) indicate that a high number of growth-retarded fetuses cannot be diagnosed by this method As intrauterine foetal growth retardation secondary to placental insufficiency is responsible for a significant proportion of perinatal mortality in modern obstetrics it would be of great clinical importance to predict this condition more accurately In a previous report (9) it was concluded that serial CAP determinations would be a valuable parameter in judging the condition of the placenta the significance of CAP assays however could not be established until the method was compared with other tests i e urinary oestriol excretion, on the same clinical material

In the present study a poor correlation was found between CAP and oestriol assays in *normal* pregnancy This indicates that the two methods are measuring quite different parameters On account of the complex aetiology of intra-uterine growth retardation this means that serial CAP and oestriol assays may give different results depending on the cause of the pregnancy at risk This is also found in the present study (Fig 5) where the CAP levels were decreasing in 3 patients whereas the oestriol excretion during the same time increased in two patients and was stationary in one Among these patients two intrauterine fetal deaths occurred and one patient had to be delivered by cesarean section because of fetal distress caused by placental insufficency Contrary to this in two patients with moderate pre-eclampsia the CAP levels were rather low whereas the oestriol excretion was within the normal range They delivered normally and the weights of the babies were normal In one patient with severe pre-eclampsia very high CAP levels and low oestriol excretion was found Pronounced degenerative changes were observed in the placenta and in this case the CAP levels must have been misleading These observations indicate that CAP and oestriol are measuring different parameters, that one test cannot substitute for the other but that they are complementary The same conclusion has been made by Petrucco et al. (6) and Tovey et al (7) Tovey et al stated that serial CAP and oestriol estimations correlated well with the clinical state in approximately 60 % of complicated pregnancies. Petrucco et al. concluded that CAP estimations were superior to oestriol assays for the prediction of intra-uterine foetal growth retardation

From the results presented in this study it is clear that single estimations of CAP are of limited value unless very low values are obtained Thus in two patients with moderate pre-eclampsia (Fig. 2) the CAP levels were below the 10th percentile but no signs of dysmaturity were observed at birth. The CAP values of the patients presented in Fig 5 were all within the normal range but decreasing values were observed during at least 14 days preceding intrauterine asphyxia. Thus serial assays with rising CAP levels are in favour of normal placental function whereas decreasing CAP levels indicate poor placental function However even in normal pregnancy there is a tendency to decreasing CAP levels after 40 weeks of gestation (8) The half-life of the enzyme in blood is rather long about 5 days (9). This implies that changes in placental function are reflected rather slowly in the blood and restricts the merit of the method

Previous comparative studies (7 6 3) concerning CAP and oestriol assays in assessing the feto-placental unit have shown that serial CAP assays give at least as reliable information as oestriol assays in predicting the foetal outcome The present study shows that serial CAP assays can give important information in forecasting the fetal outcome where the oestriol assays fail to do so and *vice versa* The conclusion can be made that both tests are needed in order to get a more diversified picture with respect to the condition of the fetus and placenta and thus constitute a better basis for dealing with the individual patient Recently an automated method for serial estimations of CAP has been presented (3) which means that the analysis can be performed rapidly and cheaply CAP assays can therefore be used as a simple and reliable means of large scale monitoring of normal and abnormal pregnancies during the last trimester

ACKNOWLEDGEMENTS

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REFERENCES

1. Bjerø, K.: Oestriol assay in obstetric practice. Acta Endocrinol (Kbh) 69 Suppl. 161 1972.
2. Booth, R. T. Stern, M. I., Wood, C. Sharples, M. J. H. & Parkerton, J. H. M.: Urinary oestriol as an index of placental function and foetal viability. J Obstet Gynaecol Br Commw 71 266, 1963
3. Chapman, L., Silk, E., Skupny A., Tooth, E. A. & Barnes, A. Spectrofluorometric assay of serum cystine aminopeptidase in normal and diabetic pregnancy compared with total oestrogen excretion. J Obstet Gynaecol Br Commw 78. 435 1971
4. Curzen, P. & Varma, R. A comparison of heat stable alkaline phosphatase and urinary oestrogen excretion in the mother as placental function tests. J Obstet Gynaecol Br Commw 78 606, 1971
5. Hainsworth, I. R. & Hall, P. E. A simple automated method for the measurement of oestrogens in the urine of pregnant women. Clin Chem Acta 35 201 1971
6. Petrucco, O. M., Cellier K. & Fishtall, A.: Diagnosis of intrauterine fetal growth retardation by serial serum oxytocinase urinary oestrogen and serum heat stable alkaline phosphatase (HSAP) estimations in uncomplicated and hypertensive pregnancies. J Obstet Gynaecol Br Commw 80 499 1973.
7. Tovey J. E., Sykes, J. R., Robinson D. A., Harry D. J., Cooper K. & Beynon, C. L.: Comparison of total urinary oestrogens and serum cystine aminopeptidase estimation in pregnancy. Clin Biochem 5 104 1972.
8. Rydén, G. Cystine aminopeptidase activity in pregnancy. A rapid method for estimations of placental function in clinical practice. Acta Obstet Gynecol Scand 50 253 1971
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MEDICAL AND SOCIAL ASPECTS OF ADOLESCENT PREGNANCIES

I *Adolescents Applying for Termination of an Illegitimate Pregnancy*

O Widholm Riitta-Liisa Kantero and Elma Rautanen

From the Department of Obstetrics and Gynaecology (Head Acting Professor Olof Widholm), University of Helsinki, Helsinki Finland

Abstract The socio-medical aspects of 131 abortion patients under the age of 18 were analysed. The following results were obtained The social background and school education of this group of abortion patients is somewhat above average compared with the general population in Finland The family of the subjects is incomplete more often than is the case in the average Finnish population (34.3% compared with the average of 14.4%). The girls were more often illegitimate and more parents are divorced than expected The relationship between the subject parents is often seriously disturbed (32.6%) Ill health is frequent among the mothers of the subjects (23.7%) The home environment seems to have lacked security The subjects are sexually relatively well informed 87.8% stated they understood the consequences before their first intercourse An attempt to prevent pregnancy was made in only 35.9% The subjects began sexual intercourse earlier than is common in Finland. 37.1% before the age of 15 compared with 6.6% in general and had several partners 66.5% compared with 11% in general In many cases there was positive emotional involvement of the subjects with their partners. Abortion is used as a means of prevention 80.9% decided to have abortion immediately Pregnancy is discovered early and medical services are effectively used 11.4% arrived at the hospital after the 13th week of gestation 29.7% of the parents did not know about the abortion 33.6% of the subjects were under the influence of alcohol at the time of intercourse, none of the girls was under the influence of drugs This group of patients are in need of continuous medical and social counselling

There has been a movement in several European countries and in North America during the second half of the 1960s towards more liberal legislation with regard to abortion This was preceded by a period of increased sexual freedom which was connected with general technical and ideological

According to the Finnish Abortion Act of 1970 an abortion can be performed without a doctor's certificate on women under 17 and over 40 years of age. For women between 17 and 40 a medical certificate is needed but abortion can be performed on social indications which include illegitimate pregnancy No consent from the guardian is required for abortion if the patient is a minor i.e a girl under the age of 20 No counselling is available for those applying for an abortion.

When the restrictions on abortion were removed in Finland little attention was given to the possible consequences It was hoped that the new legislation would eliminate the need for illegal abortions On the other hand the risk could not be ignored that easy access to abortion might make it a substitute for contraception, particularly as it did not seem that the propagation of birth control was sufficiently effective

The main purpose of this investigation was to establish whether girls under 18 resort to legal abortion as a means of contraception. In addition the physical maturation and the social status of these patients were analysed

MATERIAL AND METHODS

The series consists of patients treated at the Department of Obstetrics and Gynaecology Helsinki University Central Hospital, which mainly serves Helsinki and its immediate surroundings All girls under 18 admitted for abortion during the period July 1 197[illegible] Feb 28 1973 were included in the study The total number of such patients was 131

The girls were asked to fill in two questionnaires One

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MEDICAL AND SOCIAL ASPECTS OF ADOLESCENT PREGNANCIES

I. *Adolescents Applying for Termination of an Illegitimate Pregnancy*

O. Widholm, Riitta-Liisa Kantero and Elina Rautanen

From the Department of Obstetrics and Gynaecology (Head: Acting Professor Olof Widholm), University of Helsinki, Helsinki, Finland

Abstract. The socio-medical aspects of 131 abortion patients under the age of 18 were analysed. The following results were obtained. The social background and school education of this group of abortion patients is somewhat above average compared with the general population in Finland. The family of the subjects is incomplete more often than is the case in the average Finnish population (34.3% compared with the average of 14.4%). The girls were more often illegitimate and more parents were divorced than expected. The relationship between the subject's parents is often seriously disturbed (32.6%). Ill health is frequent among the mothers of the subjects (33.7%). The home environment seems to have lacked security. The subjects are sexually relatively well informed: 67.8% stated they understood the consequences before their first intercourse. An attempt to prevent pregnancy was made in only 35.9%. The subjects began sexual intercourse earlier than is common in Finland: 37.1% before the age of 15 compared with 0.6% in general, and had several partners: 66.5% compared with 11% in general. In many cases there was positive emotional involvement of the subjects with their partners. Abortion is used as a means of prevention: 80.9% decided to have abortion immediately. Pregnancy is discovered early and medical services are effectively used. 11.4% arrived at the hospital after the 13th week of gestation. 25.3% of the parents did not know about the abortion. 33.6% of the subjects were under the influence of alcohol at the time of intercourse; none of the girls were under the influence of drugs. This group of patients are in need of continuous medical and social counselling.

There has been a movement in several European countries and in North America during the second half of the 1960s towards more liberal legislation with regard to abortion. This was preceded by a period of increased sexual freedom, which was connected with general technical and ideological evolution.

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The girls were asked to fill in two questionnaires. One

Table I *Menstrual patterns in the abortion patients and in a control group of the same age*

	Abortion patients	4 0–4 9 years menstruating girls in the control group (21)
Menarche	12.3 years	13.. years
Regular menstruation	77 1%	76 0%
Normal duration of menstrual flow (4–7 days)	95 4%	93 7%
Normal period (22–33 days)	90 1%	85.3%
Premenstrual oedema	13 9%	10 4%
Repeated dysmenorrhoea	37 4%	18 2%

contained questions concerning growth time of first menstruation and menstrual pattern before the onset of pregnancy It was completed under the supervision of a medical social worker The questionnaire was later supplemented by data obtained from the hospital records on the method of abortion and on any subsequent complications

The other questionnaire was designed to record information on the patient s social and family background personal relationships within the family her sexual behaviour and partners. This questionnaire was filled in by the patient alone In order to facilitate statistical analysis of the material closed questions were used Confidential treatment of the data was guaranteed and the questionnaire was returned in a sealed envelope The statistical analysis was performed at the Helsinki University Computing Centre

According to the hospital routine abortion was invariably induced by the vaginal route if the duration of pregnancy was under 13 weeks. At later stages either prostaglandin or hysterotomy was used

RESULTS

Somatic maturation

The mean chronological age of the patients was 16 8 years (S D 0 9 years) There were no patients under 14 4 6% were 14-year-olds 10 7% 15-year olds 36 6% 16-year-olds and 48 1% 17 year-olds The mean age at menarche was 12.3 years (S D 1 1 year) The mean height of the patients was 163 0 cm (S D 5 9 cm) at the time of menarche it was 155 5 cm (S.D 6 8 cm)

The endocrine maturation of young women is to a great extent reflected by their menstrual pattern In Table I the results in this series are compared with the values previously obtained from sample of the general population (21) In the present series the average time from the first menstruation was 4 5 years Therefore girls who had menstruated for 4 0–4 9 years were chosen as a control group As may be seen in the table, the age at menarche was significantly lower ($P<0\ 001$) the number of normal periods was higher (P=N S) and the frequency of premenstrual oedema (P= N S) and recurrent dysmenorrhoea was significantly higher ($P<0\ 01$) in the abortion series than in the control group (9 21)

Methods used in termination of pregnancy and complications

The methods by which abortion was induced in the present series appear in Table II The majority of the patients were admitted before the end of the 13th week of gestation only 15 girls came to the hospital at a later stage When the patients were asked how long their menstrual period had been overdue when a suspicion of pregnancy arose the average answer was 2 6 weeks The pregnancy was diagnosed on average 4.5 weeks after the first missed period Abortion was induced after an average duration of pregnancy of 11.3 weeks (S D 3 0 weeks)

The proportion of complications after the abortion was 18 5% 16 2% or 21 cases had a temperature rise of up to 38°C after the emptying of the uterus and were diagnosed as post abortion endometritis cases In ? of the cases a positive culture of Neisseria gonorrhoea was obtained In 3 cases the bleeding during the D&C was over 500 ml and in two of these cases a repeat curettage was performed A blood transfusion was indicated in one of the cases belonging to the group of infections after the treatment

SOCIAL AND FAMILY BACKGROUND

The Helsinki University Clinic draws most of its patients from the city of Helsinki and its immediate

Table II *Methods of abortion (no 131)*

Method	Patients (%)	Gestation week
Dilatation and suction	64 3	<13
Dilatation and curettage	24 0	<13
Prostaglandin infusion and curettage	8 5	>13
Abdominal hysterotomy	3 1	>13

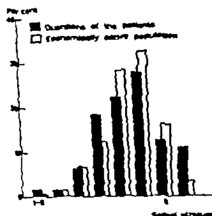


Fig. 1. The distribution of the patients in the social strata

environment. Therefore all the subjects of this study come from industrialised areas. 72.5% had lived in urban communities and 27.5% in rural communities where, however, the degree of industrialisation was comparatively high. All subjects were Finnish citizens and none of them belonged to a foreign race. With a few exceptions they belonged to the protestant Lutheran church, the main church in Finland.

At the time of the abortion, very few of the subjects had reached a social position of their own. It was therefore assumed that their distribution into the different strata of society was the same as that of their guardians. The distribution was made according to the scale of Rauhala (13, 14). As seen from Fig. 1, the subjects are overrepresented in the five highest strata and again in the last or ninth stratum of society. Thus the abortion patients could be divided into three groups: those coming from the five highest strata of society, those coming from the next three strata and those coming from the lowest stratum of society. The difference between the guardians of the subjects and the population in Finland in regard to their distribution in the social strata is statistically significant at a high level ($P<0.001$).

The following comparison can be made between the social background of the subjects and that of the average population. The impression that the majority of the subjects did not belong to the lowest strata of society is further strengthened by the in-

Rauhala divides the population into 9 strata according to how their occupation is valued in society.

Table III. *Social background*

	Subjects (%)	Average population* (%)
More than compulsory school education	33.6	24.0
Parents had more than compulsory education	14.5	11.0
Crowded housing	19.9	14.0
Incomplete family	34.5	14.4

* References 6, 18, 19

formation concerning their school education as well as that of their parents, as shown by Table I. It is true that the subjects came from highly industrialised areas where the opportunities for higher school education are good. Even then it can be said that the average basic education of a majority of the subjects as well as that of their parents is somewhat higher than average in Finland.

Housing conditions in the homes of many subjects were crowded. Of all subjects, 19.9% had lived in homes where there were more than two persons per room. In 1960, the corresponding figure among the population in general was 14% (18). There is a high correlation (0.259) between the stratum of society to which the subject belongs and the housing conditions. Crowded housing is mainly centred in the lowest strata.

As seen from Table III the proportion of the subjects who had grown up in incomplete families was very high compared with the average population. Moreover among the families classified as complete there were a few step-fathers and step-mothers, indicating that there had been either an originally incomplete family or that the original family had been broken up by death or divorce.

Even when there was a complete family, the relationship between the parents was often poor. Constant quarrels and fights were reported in 32% of the cases. There is a high correlation (0.217) between the social stratum and the relationship between the parents. This does not, however, necessarily mean that parental disharmony was entirely confined to the lowest strata. It is likely that the parents in these strata are used to expressing themselves more freely in front of their children. The disharmony of the parents was frequently accompanied by the mother's lack of interest in the daughter (0.318).

Table IV *Sexual behaviour*

	Subjects (%)	Other studies* (%)
Sexual intercourse before 15 years of age	32.1	0.6
Sexual intercourse before 16 years of age	68.0	4.5
More than one partner	66.3	11 0

References 17 22

In four cases the mother had died before the daughter had started school In 23 7 % of the cases where the mother had been alive she was reported to have suffered from frequent or chronic ill health There is a very high correlation (0 490) between the health of the mother and her interest in the daughter The patients whose mothers were frequently ill felt that their mothers were not interested in them

There emerges a picture of family instability due to parental divorce or illegitimate birth Frequent illness of the mother was reported in a great number of cases and also complete disharmony between the parents One or several of these factors *which may* contribute to a disturbance in the family and in the early human relationships of the subjects were present in many cases Thus the home environment of the subjects often lacked safety and security

SEXUAL BEHAVIOUR

It is common to refer to the lack of sexual information as one significant reason for early illegitimate pregnancies (3 4) Of this group of subjects 13 7 % reported that they had got their sexual information from experience and 87 8 % stated that they had understood the consequences *before their first* intercourse

The sexual behaviour of the subjects can be clarified by the following comparison (Table IV) It can be seen that many more of the subjects had started to have sexual intercourse at an earlier age than is the case among the *female population* in Finland in general Also the subjects had more often had several partners There were only eight cases of cohabitation and in nine cases the partners were engaged Twelve of the subjects reported that they had not known their partners at all The sexual relationship which resulted in pregnancy and abortion had lasted over *an average period* of eight months Most of the subjects reported that they had been in love with their partners Thus there was a positive emotional involvement with the partner Five of the subjects stated that they intended to get married to their partners In almost all cases both partners had been equally active in the initiation of the intercourse which resulted in pregnancy and abortion

In a majority of the cases the male partner in the crucial intercourse was unmarried and in 70 % he was reported to be older and more experienced than the girl Of the cases where information of the exact age of the partner was given it appeared *that the difference of age was generally only a few* years but in a few cases it was 10 years or more

Although most of the subjects knew about means of prevention an attempt to prevent pregnancy was made in only one third of the cases (35 9 %) With the exception of one case where the girl was on *the Pill it was the male partner who had attempted* prevention using a condom In half of the cases sexual life between the partners had been regular but for 15 of the subjects (11 3 %) it was their first experience of sexual intercourse

About four-fifths of the patients had had their *pregnancy confirmed by a doctor before the end of* the second month and 88 4 % of them came to the hospital for abortion before the 13th week of pregnancy

Two thirds (66 4 %) of the subjects used alcohol *most of them rarely The great majority maintained that they did not use drugs During sexual inter*course only 33 6% of them were under the influence of alcohol but none of them under the influence of drugs In a quarter of the cases (25 ?%) the parents of the patients did not know about the abortion In another nine cases the parents *were indifferent or cut off their relationship* with the daughter

DISCUSSION

In July 1970 a more liberal aborti n act wa passed in Finland the most essential inn vati n of which was that unfavourable social c nditi n including being unmarried were accepted a an indication *for the termination f pregnancy Girl under* 17 years of age ar granted aborti n without a d c tor s certificate during the first 16 weeks of gestation A tati ti s of the Medical Board f r 1971–

1972 are not yet available we do not know with certainty whether the frequency of induced abor tions in adolescents has risen as a consequence of the new legislation The statistical data for our hospital indicate that there has been an even greater increase in induced abortions in the younger than in the older age groups. Moreover it is known that also at private hospitals terminations of pregnancy in young women are performed in great numbers. Whether this increase implies that previously illegal abortions are now legitimately per formed, or that pregnancy is more common than before in adolescents, remains an open question. In any event the fact that 130 young girls applied for abortion at one hospital within about half a year shows that a serious problem is involved.

One purpose of the present investigation was to ascertain whether there are any special circumstances or factors characteristic of girls becoming pregnant at an early age does their home back ground differ from the average and have they re ceived adequate and pertinent sexual instruction what would be the best approach to the sexual social, and associated problems of these adolescents?

It has been previously established that the endocrine development of young girls is slow an adult menstrual pattern is only attained after 5 years menstruation according to Vollman (20) it may not be attained until 10 years from menarche. The increase in height after the menarche has been indicated at an average of 7 4 cm (16) By the ad ministration of oestrogens during puberty it is possible to arrest growth earlier than normal, and perhaps also to diminish height increase. Consequently increased oestrogen secretion may have a depressiv effect on the growth of pregnant girls of pubertal age The mean height in the present series was 163 0 cm In the general population the mean height of girls of the corresponding age groups is 164 8 cm (1) The difference 1.8 cm, is statistically significant ($P<0\ 01$). On the other hand the mean height of the present abortion patients at the time of menarche was 155 5 cm, which is 1.5 cm les than in the control group (8) The average height increase after menarche was thus the same in both groups, or 7.5 cm which corresponds to the alues seen in the literature on this subject The bortion patient seem to represent an early maturing type of girls, who start menstruating earlier and are maller than the average at the age of 17 Whether these girls miss the slight height increase normally occurring after the age of 17 (16) remains an open question since the series was not followed up after abortion

With regard to regularity of the menstrual cycle and duration of menstrual flow there was no dif ference between the series investigated and the control group of girls who had menstruated for 4 years (21). By contrast, the frequency of premenstrual tension was 3.5% higher in the abortion group and dysmenorrhoea was almost twice as common as in the control group (21) With regard to these features the abortion group was more like an adult control group than the control group of girls of the same ages The impression that the patients represented a physically early maturing type is thus strengthened

In the adult patient material the morbidity rate of legal abortions in our hospital is around 13% Compared with this number an 18.5% frequency of complications is significantly higher Also there were two cases of gonorrhoeal infection, and in one case a suspected perforation of the uterus. In an investigation on pregnancy terminations induced with prostaglandin, rupture of the uterus was found to occur in 5 out of 100 of patients under 20 years (7). All these patients were nulliparae aged 15–19 Although pregnancies of over 13 weeks duration constituted only 11 4% of the present series the possibility of clinical complications is always a fac tor to be taken into account. The later treatment of the complications is frequently a problem, be cause the patients have little personal experience of gynaecological symptoms and are not used to consult a gynaecologist after discharge from hospital Ideally the follow-up examination should be a combined medical and social consultation. There should be a possibility for continued visits and counselling

As to the social background of the patients it is somewhat above average compared with the general population in Finland. This finding differs from those of Kaila (10) and Paavola (12) The family of the group deviates clearly from that of the aver age population n that 34% of the patients came from incomplete families with no father figure (2) In a third of the cases there was a serious marital conflict between the parents of the patients. Ac cording to study by Sievers et al (17) the same situation was found in 23% of [illegible] erage Finnish families Of the mothers of the patients 23% were

reported to have suffered from frequent or chronic ill health It is not possible to compare this figure with any available statistics because the basis of the estimate varies In spite of this the proportion of mothers having poor health seems high

Crowded housing conditions in the homes of many patients is probably one reason why the young people spend as much time as possible out side their own home This again would be likely to cause feelings of loneliness and need of protec tion an impression which is further strengthened by the fact that the majority of the patients had seen their male partners as older and more expe rienced than themselves and claimed to be in love with them. Moreover 25% of the girls had not been close enough to their parents to let them know about the pregnancy and abortion In addi-tion to this parents were indifferent or hostile in 7% of the cases It has been suggested (15) that in cases where communication between family members has failed and as a result the subject has difficulties in communicating with her environ-ment on an emotional level sexual intercourse is an attempt to communicate on the physical level It is frequently maintained that the sexual problems of young people are due to the lack of sexual in-formation. Of the girls in this study 88% knew about the risk of becoming pregnant as the result of sexual intercourse This finding supports the opinion expressed by Deutsch (5) that lack of sexual information has nothing to do with adoles cent pregnancies The subjects of this study were sexually well informed before the beginning of the pregnancy which resulted in abortion In spite of this only 36% had attempted to prevent it during the intercourse It does not seem that the infrequent use of contraceptives is attributable to the use of alcohol or drugs

The girls of this study had their first experiences of sexual intercourse very early 37% of them under the age of 15 compared with 0 6% (17) among the average female population In our study 67% of the girls had had more than one partner In the study of Winberg (22) the corresponding figure was 40% In spite of the frequent changing of partners the sexual relations with the partners who caused the pregnancy had lasted over a period of 8 months This finding coincides that of Kinch (11).

This study shows clearly that subjects used abor tion as a means of prevention in a way not intended by the law

The contention that abortion is resorted to as a substitute for contraceptive measures is also proved by the fact that since 1970 girls from 15 to 16 years of age have been seen in our hospital even for the third time asking for abortion

It seems that the liberal law on abortion has led to unforeseen consequences and it has not there fore been possible to arrive at any accurate deci-sion as to what actions should be taken in the pre-ventive and post abortion treatment of young abor tion patients

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REFERENCES

1 Bäckström, L. & Kantero R. L. Cross-sectional studies of height and weight in Finnish girls aged from birth to 20 years Acta Paediat Scand Suppl. 220 9 1971
2 Barglow P Bornstein, M Exum D B Wright, M K & Visotsky H M Some psychiatric aspects of illegitimate pregnancy in early adolescence. Amer J Ortopsychiat 38 672, 1968.
3 Cobliner W D Schulman H & Seymour L. The termination of adolescent out-of wedlock pregnancies and the prospects for their primary prevention Amer J Obstet Gynecol 115 432, 1973
4 Connel, E. B & Jacobson L. Pregnancy the teen-ager and sex education. Am J Public Health 61 1840 1971
5 Deutsch H Selected problems of adolescence, p. 107 International Universities Press Inc New York, 1967
6 Haavio-Mannila, E Suomalainen nainen ja mies. s 44 Werner Söderström Porvoo, 1968.
7 Kajanoja, P Jungner G Widholm O Karjalainen O & Seppälä M Rupture of the cervix in prosta-glandin abortions. J Obstet Gynecol Br Emp, 1973 In press.
8. Kantero R. L. Unpublished material
9 Kantero R. L. & Widholm O The age of menarche in Finnish girls in 1969 Acta Obstet Gynecol Scand. Suppl 14 7 1971
10 Kaila O Poikkeukaellisen nuorina synnyttäneistä Thesis Helsinki 1968
11 Kinch R A Social aspects of pregnancy in adoles-cence Exc Medica 279 210, 1973
12. Paavola A The illegitimacy rate and factors in-fluencing the pregnancy and delivery of unmarried mothers. Acta Obstet Gynecol Scand Suppl 3 p 67 1968
13 Rauhala, U Suomalaisen yhteiskunnan sosiaalinen kerrostuneisuus Werner Söderström Porvoo 1966 Annex
14 — Suomalaisen yhteiskunnan sosiaalisten kerrostu-

mien määrällinet vaihtuudet. Sosiaalinen Aikakauskirja 6 1 1969
15. Rantanen E. Avioton äiti ja adoptiopäätös (The unmarried mother and the adoption decision), p. 117 WSOY Helsinki 1972.
16. Roche A. F & Davila, G H Late adolescent growth in stature Pediatrics 50 874 1972.
17. Sievers, K Koskelainen O & Leppo, K Suomalaisten sukupuolikäyttäytyminen. Werner Söderström, Helsinki 1973
18. Sauna, L. Asuntopolitiikan kysymyksistä. Asuntoforum 2 15 1969
19. Suomen Tilastollinen Vuosikirja, 1966
20. Vollman, R. F Pattern of menstrual performance in adolescent girls. Proc Sec Wld Congr Fertil Steril, p. 27 Naples, 1956.
21. Widholm, O & Kantero, R.-L. Menstrual patterns of adolescent girls according to the chronological and gynecological ages. Acta Obstet Gynecol Scand, Suppl. *14* 19 1971
22. Wiaberg, B Sexuellt beteende bland finlandssvensk ungdom. Sosiologia 2 80 1967

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CALF BLOOD FLOW AND VENOUS CAPACITY DURING LATE PREGNANCY IN WOMEN WITH VARICES

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Abstract Venous volume, capillary filtration rate and blood flow were determined by plethysmographic methods in the legs of three different groups of women. Groups I and II consisted of women in late pregnancy with and without varices of the legs. Group III consisted of nonpregnant, nulliparous women. Venous volume with correction for the capillary filtration is markedly larger in the pregnant group with varices (Group I) than in the two other groups (Groups II and III). Resting blood flow is markedly larger in Group I than in Group II and slightly larger in Group I than in Group III. Also peak blood flow is larger in Group I than in Group II. No significant differences are found in capillary filtration rate. In Groups II and III there is a significant positive correlation between the venous volume and the resting blood flow and peak blood flow. Such a correlation does not exist in the varix group (Group I). The result supports the theory by Haeger (7) that in pregnant women arterio-venous anastomoses contribute to the development of varicose veins.

In primigravid women without varices venous volume in the calf expressed in ml/100 ml tissue and capillary filtration rate expressed in ml/min/100 ml tissue are not increased compared with non-pregnant women (11). Resting blood flow is [illegible] in primigravidas than in the non-pregnant state, most markedly in the third trimester (13). These results are based on measurements performed with plethysmographic method with the subjects in the supine position, a position where the pregnant uterus compresses the large vessels to and from the pelvis and the legs. The compression as well as the haemodynamic effect of the placenta (2, 3, 13) gives rise to a high venous pressure in the legs. This might be one of several factors that contribute to the development of varices. Another aetiological factor seems to be a hereditary weakness of the vein wall (16, 17).

In non-pregnant subjects with varices Eiriksson & Dahn (5, 6) found a larger venous volume than in a control group without varices. Capillary filtration rate was however the same in both groups. Like the findings of Aremander (1) no difference in resting blood flow was found, and this was taken as an argument against primary varices being caused by congenital arterio-venous fistulae as suggested by Piulachs & Vidal-Barraquer (10).

Haeger postulated that during pregnancy arterio-venous anastomoses in the calf presumably contribute to the development of varicose veins (7).

The aim of this study was to measure and compare some circulatory parameters in the legs of non-pregnant women and pregnant women with and without varices. If Haeger's postulation is true pregnant women with varices would have higher blood flow in the calfs than pregnant women without varices. Moreover capillary filtration rate in the leg during pregnancy has not been measured earlier.

MATERIAL

Seventeen pregnant women with moderate to large superficial varices of the legs were investigated. There were no signs of venous thrombosis or insufficient perforants. Twenty-two pregnant and 22 non-pregnant, nulliparous women without varices were used as control groups. Details of the groups are given in Table I.

METHODS

All measurements were done by means of water-filled venous occlusion plethysmograph of a type earlier described (4, 12). The procedure of blood flow measurement in resting conditions and during reactive hyperaemia (peak blood flow) were the same as used by Sandström (12).

Table I *Age number of pregnancy duration of pregnancy volume of calf segment and haemoglobin concentration of the three groups investigated* (Mean ± S D)

	Group I (n = 17)	Group II (n = 12)	Group III (n = 12)
Age years	28.9±5 0	23 4±3 4	18.8±3 3
Pregnancy numbers	2 7±1 3	1 0±0.0	-
Duration of pregnancy weeks	37 8±1.3	38 0±1 0	-
Calf segment volume ml	1 324±181	1 378±185	1 272±165
Haemoglobin concentration, g%	11 1±1 0	11 6±0.6	12.1±0.8

Capillary filtration rate was determined by a method modified from Mellander (9) and Spetz (14) and the net filtration was expressed in ml·min 100 ml⁻ tissue At cuff pressures on the thigh below 40 mmHg the net filtration was considered so slight that it has been neglected It is assumed that at rest 80% of the applied occlusion pressure is reverted to the capillary level (9) As the mean water pressure from the plethysmograph on the calf is 10 mmHg the remaining lateral venous pressure will be below 22 mmHg which is probably lower than the colloidosmotic pressure Moreover there is a linear relationship between the lateral venous pressure and the net capillary filtration (8)

The slight rise of the plethysmographic volume curve after the first rapid rise at cuff pressures higher than 40 mmHg was regarded as the net filtration

Venous volume was determined by a modification of the method used by Sandström (12). At cuff pressures higher than 40 mmHg there is no distinct beginning of the second slow-rising phase of the plethysmographic recording representing a constant venous volume and constant filtration rate Moreover it is often difficult to obtain a stable base line because of small unavoidable leakage in the air-conducting system of the plethysmograph and a hysteresis effect on the veins. Therefore some corrections and simplifications were made (see Fig. 1) At cuff pressure 40 mmHg one line is drawn through the first steep rise of the curve and one line through the second stable part of the curve Assuming no filtration to take place the perpendicular distance between the crossing point of the two lines and the base line is said to be the venous volume at cuff pressure 40 mmHg (= V_{40}) At the volume curves recorded with cuff pressures 60 and 80 mmHg, V is marked by a perpendicular line Where the line crosses the base line (time axis) the lateral venous pressure is assumed to override the colloid osmotic pressure and filtration begins. From this point on the base line a straight line is drawn parallel to the slow-rising second part of the curve The perpendicular distance between these parallel lines is measured It represents the venous volume at cuff pressure 60 and 80 mmHg respectively It is likely that the constructed oblique base line sometimes gives an overestimation of the filtration at the beginning and consequently a slight underestimation of the venous volume but the possible error is small because the calf volume increase due to filtration is small compared to the venous volume

The error of a single measurement was calculated from twelve double determinations performed on two consecutive days. In determinations of the venous volume this error was found to be 17–22% (the higher value applying to a venous occlusion pressure of 60 mmHg) The error of capillary filtration rate was 9

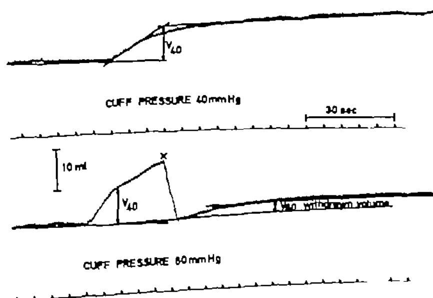


Fig 1 Venous volume at occlusion pressure 40 mmHg and 60 mmHg respectively The marked drop in the curve at × due to with-draw l of water from the plethysmograph. (For further explanation see text)

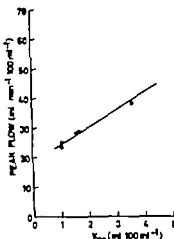


Fig 2. Relationship between venous volume (V_{80}) and peak flow in non-pregnant multiparous women (Group III). The equation of the regression line is $y = 0.59x + 1.83$ with residual variance $= 0.66$.

52% (the higher value applying to venous occlusion pressure of 60 mmHg).

Haemoglobin concentration was determined in peripheral blood at the time of the plethysmographic examination. The determinations were performed spectrophotometrically at wavelength of 544 mμ (oxyhaemoglobin) after adding 0.04% ammonium hydroxide. The error of single measurement was calculated from twelve double determinations from fingertip blood and found to be 1.0%.

Regression equations standard deviation (S.D.) and correlation coefficients (r) were calculated according to the method of least squares. The differences and the correlations were tested by the Student t-test and the probability (p) was indicated at one of the following three levels: $0.05>p>0.01$ (or almost significant), $0.01>p>0.001$ (or significant), and $0.001>p$ (or highly significant).

RESULTS

The age of the pregnant women with varices (Group I) is higher than that of the other two groups and this group of women with varices has also undergone a greater number of pregnancies. There is no significant difference between the examined calf segments in the three groups. The haemoglobin concentration is however lower in the pregnant group with varices (Group I) than in the non-pregnant group (Group III), but not in the pregnant group without varices (Group II) (Table I).

For comparisons between the groups the individual mean value for both legs was used. The same mean values were also used in calculating regression equations and correlation coefficients. In the varices group both resting- and peak blood flow is higher than in the pregnant group without varices. In comparison with the non-pregnant group the varices group has higher resting blood flow but the difference is small ($0.05>p>0.01$). Venous volume is markedly larger in the pregnant group with varices than in the other two groups between whom no difference is noticed. In groups II and III there is a significant positive correlation between the venous volume and the resting blood flow and peak blood flow (Fig. 2). Such a correlation does not exist in the varix group (Group I). Capillary filtration rate is the same in all three groups. For further details see Tables II and III.

DISCUSSION

The average age of the pregnant women with varices is higher than that of the women in the

Table II. *Circulatory parameters studied by venous occl sion plethysmogr phy d ring pregnancy ith (Group I) and ithout arices (Group II) d in a reference group (Group III)* (Mean ± S.D.)

	Group I (n 17)	Group II (n 22)	Group III (n=22)	Diff I–II	Diff I–III	Diff II–III
Resting blood flow ml min⁻¹·100 ml⁻¹ tissue	4.8±2.4	2.1±0.7	3.1±1.6	7**	1.7	−1.0
Peak blood flow ml min⁻¹·100 ml⁻¹ tissue	37.8±11.6	28.0±7.0	34.3±9.9	9.8	3.5	−6.3
Venous volume ml 100 ml⁻¹ tissue						
Occlusion pressure 60 mmHg	4.4±1.0	2.7±1.0	2.7±1.1	1.7	1.7***	0
Occlusion pressure 80 mmHg	5.1 1.1	3 0.9	3 ±1.2	1.9*	1.9*	0
Capillary filtration rate ml min 100 ml⁻¹ tissue						
Occlusion pressure 60 mmHg	0.13+0.11	0.11±0.07	0.1 ±0.05	0.02	0.01	−0.01
Occlusion pressure 80 mmHg	0.?7 0.14	0.20±0.10	0.20±0.07	0.07	0.07	0

$0.05>p>0.01$ $0.01>p>0.001$ $0.001>p$

Table I *Age number of pregnancy duration of pregnancy volume of calf segment and haemoglobin concentration of the three groups investigated* (Mean ± S D)

	Group I (n=17)	Group II (n=22)	Group III (n=22)
Age years	28 9±5 0	23 4±3 4	18 8±3 3
Pregnancy numbers	2.7±1.3	1 0±0 0	–
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Calf segment volume ml	1 374±181	1 378±185	1 272±165
Haemoglobin concentration, g%	11 1±1 0	11 6±0 6	1_1±0.8

Capillary filtration rate was determined by a method modified from Mellander (9) and Spetz (14) and the net filtration was expressed in ml/min 100 ml⁻¹ tissue. At cuff pressures on the thigh below 40 mmHg the net filtration was considered so slight that it has been neglected. It is assumed that at rest 80% of the applied occlusion pressure is reverted to the capillary level (9) As the mean water pressure from the plethysmograph on the calf is 10 mmHg the remaining lateral venous pressure will be below 2_ mmHg which is probably lower than the colloidosmotic pressure Moreover there is a linear relationship between the lateral venous pressure and the net capillary filtration (8)

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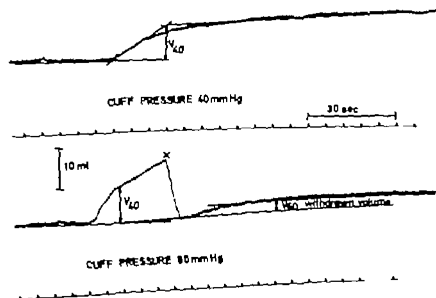


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AMNIOTIC FLUID PHOSPHOLIPID DETERMINATIONS BY BUBBLE STABILITY TEST AND QUANTITATIVE LIPID ANALYSIS

T Lindback, J Skjeraasen and L Caicedo

From the Pediatric Research Institute (Head M Seip) and the Department of Obstetrics and Gynecology (Head K Bjøro), Rikshospitalet Oslo Norway

Abstract Amniotic fluid surfactant content has been determined in 60 samples obtained throughout the last trimester by the bubble stability test (BST) described by Clements et al. (3), and studied quantitatively by measuring lecithin and sphingomyelin. Forty samples obtained than one week of delivery were also studied in relation to gestational age and development of the respiratory distress syndrome (RDS). Bubble stability is shown to be dependent on the lecithin concentration Respiratory distress is shown to be associated with low levels of lecithin and negative BST although false negative BST may occur

Methods for determining amniotic fluid surfactant in relation to fetal pulmonary maturity have mainly consisted of phospholipid extraction and purification followed by thin layer chromatography and lipid measurement (1 4, 5 7). These methods are relatively time consuming and involve trained personnel. Clements has introduced a simple and inexpensive method for semiquantitative surfactant determination based on the ability of surfactant to *form highly stable surface films in the presence of 47.5% ethanol*. The foam generated by shaking a mixture of equal parts of amniotic fluid and 95% ethanol for 15 seconds is identified as a ring of stable bubbles in the air-liquid interface of serial dilution of amniotic fluid. A clearly positive test (undiluted 0.75 0.5 dilutions) has been suggested to indicate lung maturity and clearly negative test to indicate a high risk of RDS. Intermediate results (positive in *undiluted* or 0.75 dilutions) were considered to carry substantial risk of RDS. This has been confirmed recently by Bhagwanani et al. (2). However their report suggest that intermediate and negati test ha e a high proportion of false result I their series, 19 out of .5 cases with negati e test had normal respiration after birth. Wagstaff & Bromham (8) has compared the bubble stability test with the L/S-ratio in estimation of surfactant in amniotic fluid and concluded that the risk of hyaline membrane disease is ery small when the test is positive. When not positive they consider a chemical test necessary for accurate prediction of respiratory complications in the newborn

The present study was undertaken to determine the lecithin and sphingomyelin concentrations in amniotic fluid in relation to bubble stability de velopment of RDS and gestational age

MATERIALS AND METHODS

Amniotic fluid was obtained by transabdominal paracentesis, at caesarean section, and in some cases by amnioscopy. All samples, if not studied immediately were stored at 20°C until analyzed. The fluid was not centrifuged prior to extraction. All samples contaminated with blood or meconium were discarded. A diagnosis of RDS required the presence of respiratory distress (tachypnoea, grunting, rib retraction) beginning in the first six hours of life, persisting for more than 24 hours and of sufficient severity to require supplemental oxygen for more than 24 hours.

The lipids were determined as previously outlined (5). The concentrations of the lipid fractions were calculated in micromoles of lipid in 100 ml amniotic fluid. The test introduced by Clements was carried out and evaluated by individuals who had no prior knowledge as to the lipid content of the samples. The only modification in the procedure has been to omit the final dilution (0.20) of amniotic fluid.

RESULTS

Sixty samples of amniotic fluid obtained throughout the last trimester were tudied as outlined above. The relationship between the BST employing undiluted, 0.75 0.5 and 0.25 parts of amniotic fluid and the lecithin concentration of each individual

Table III *Correlation coefficient r_{xy} between some circulatory variables*
The same groups and the same significance as in Table II

Variables compared	Group I r_{xy}	Group II r_{xy}	Group III r_{xy}
Venous volume occlusion pressure 60 mmHg (x) – Resting blood flow (y)	0.368	0.551	0 552
Venous volume occlusion pressure 60 mmHg (x) – Peak blood flow (y)	0 444	0.639*	0.599*

other two groups investigated but as the average age in all groups is relatively low it does not seem likely that differences in blood flow and venous capacity could be ascribed to differences in age alone (15)

The larger calf blood flow of pregnant women with varices compared with pregnant women without varices and non-pregnant women may indicate the existence of arterio-venous anastomoses in this region which supports the statement of Haeger (7) who in the large saphenous vein in the prox imal part of the calf in subjects with varices found a high venous oxygen saturation and skin temperature This is also a region where varices often first become visible

The constant value of capillary filtration rate during pregnancy now found in the calf is in agreement with the findings of Spetz (14) in the right forearm during pregnancy On the assumption that the permeability of the capillary membranes in the calf and the tone of the pre-capillary sphincter vessels are unchanged during pregnancy both with and without varices the capillary area available for filtration remains constant

A possible explanation of the positive correla tion between venous volume and blood flow in women without varices may be that the size of veins is determined by arterial inflow In women with varices this normal variation in size is dis turbed

REFERENCES

1 Arenander E. Hemodynamic effects of varicose eins and results of radical surgery Acta Chir Scand Suppl. 260 1960
2 Bickers W The placenta, a mod fied arterio-venous fistula. Sth Med J 35 593 1942.
3 Bienlarz, J Crottogini J J Curuchet E. Romero-Salinas G Woshida T Poseiro J J & Caldeyro-Barcia, R Aortacaval compression by the uterus in late human pregnancy Am J Obstet Gynecol 100 203 1968
4 Dahn I On clinical use of venous occlusion plethysmography of calf I Methods and controls Acta Chir Scand 130 4.. 1965
5 Dahn I & Eiriksson E Plethysmographic diagnosis of deep venous thrombosis of the leg. Acta Chir Scand, Suppl. 398 33 1968
6 Eiriksson, E. & Dahn, I Plethysmographic studies of venous distensibility in patients with varicose veins. Acta Chir Scand Suppl 398 19 1968.
7 Haeger K Atraumatiska arterio-venösa anastomoser i benen Läkartidningen 62 1739 1965
8 Landis E. M & Gibbon J H J Clin Invest 12 105 1933
9 Mellander S Comparative studies on the adrenergic neuro-humoral control of resistance and capacitance blood vessels in the cat Acta Phys Scand 50 Suppl 176 1960
10. Piulachs P & Vidal-Barraquer F Patogenic study of varicose veins. Angiology 4 59 1953
11 Sandström B Plethysmographic studies of enous olume in the lower legs during normal primipregnancy Acta Obstet Gynecol Scand 53 97 1974
1 — Calf blood flow during normal primipregnancy Acta Obstet Gynecol Scand 52 199 1973
13 Scott D B & Kerr M G Inferior vena caval pressure in late pregnancy J Obstet Gynecol Br Comm 70 1044 1963
14 Spetz, S Capillary filtration during normal pregnancy Acta Obstet Gynecol Scand 44 .27 1965
15 Steinler H Plethysmographische Messung der Extremitatendurchblutung bei kreislauf gesunden Versuchspersonen Inauguraldissertation Tubingen, 1965
16. Zsotér T & Cronin R F P Venous distensibility n patients with varicose eins J A Med Can 94 1?93 1966.
17 Zsotér T Moore S & Keon W Venous distensibility in patients with varicosities In vitro studies J Appl Phys 22 ?05 1967

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Venous volume occlusion pressure 60 mmHg (x) – Resting blood flow (y)	0 368	0.551	0.557
Venous volume, occlusion pressure 60 mmHg (x) – Peak blood flow (y)	0 444	0 639*	0 599*

other two groups investigated but as the average age in all groups is relatively low it does not seem likely that differences in blood flow and venous capacity could be ascribed to differences in age alone (15)

The larger calf blood flow of pregnant women with varices compared with pregnant women without varices and non-pregnant women may indicate the existence of arterio-venous anastomoses in this region which supports the statement of Haeger (7) who in the large saphenous vein in the proximal part of the calf in subjects with varices found a high venous oxygen saturation and skin temperature This is also a region where varices often first become visible

The constant value of capillary filtration rate during pregnancy now found in the calf is in agreement with the findings of Spetz (14) in the right forearm during pregnancy On the assumption that the permeability of the capillary membranes in the calf and the tone of the pre-capillary sphincter vessels are unchanged during pregnancy both with and without varices the capillary area available for filtration remains constant

A possible explanation of the positive correlation between venous volume and blood flow in women without varices may be that the size of veins is determined by arterial inflow In women with varices this normal variation in size is disturbed

REFERENCES

1 Arenander E. Hemodynamic effect of varicose veins and results of radical surgery Acta Chir Scand Suppl 260 1960
2. Bickers W The placenta, a modified arterio-venous fistula Sth Med J 35 593 1942.
3 Bieniarz, J Crottogini J J Curuchet E. Romero-Salinas G Woshida T Poseiro J J & Caldeyro-Barcia, R. Aortacaval compression by the uterus in late human pregnancy Am J Obstet Gynecol 100 203 1968
4 Dahn I On clinical use of venous occlusion plethysmography of calf I Methods and controls. Acta Chir Scand 130 42 1965
5 Dahn I & Eiriksson E. Plethysmographic diagnosis of deep venous thrombosis of the leg. Acta Chir Scand, Suppl 398 33 1968
6. Eiriksson E. & Dahn I Plethysmographic studies of venous distensibility in patients with varicose veins. Acta Chir Scand Suppl 398 19 1968.
7 Haeger K Atraumatiska arterio-venösa anastomoser i benen Läkartidningen 62 1739 1965
8 Landis E M & Gibbon J H J Clin Invest 12 105 1933
9 Mellander S Comparative studies on the adrenergic neuro-humoral control of resistance and capacitance blood vessels in the cat Acta Phys Scand 50 Suppl 176 1960
10 Piulachs P & Vidal-Barraquer F Patogenic study of varicose veins. Angiology 4 59 1953
11 Sandstrom B Plethysmographic studies of enous olume in the lower legs during normal primipregnancy Acta Obstet Gynecol Scand 53 97 1974
12 — Calf blood flow during normal primipregnancy Acta Obstet Gynecol Scand 52 199 1973
13 Scott D B & Kerr M G Inferior vena caval pressure in late pregnancy J Obstet Gynecol Br Comm 70 1044 1963
14 Spetz S Capillary filtration during normal pregnancy Acta Obstet Gynecol Scand 44 227 1965
15 Steinler H Plethysmographische Messung der Extremitätendurchblutung bei kreislaufgesunden Versuchspersonen Inauguraldi sertation Tubingen 1965
16. Zsotér T & Cronin R F P Venous distensibility in patients with varicose vein J A Med Can 94 1793 1966
17 Zsotér T Moore S & Keon W Venous distensibility in patients with varicosities In vitro studies. J Appl Phy 22 705 1967

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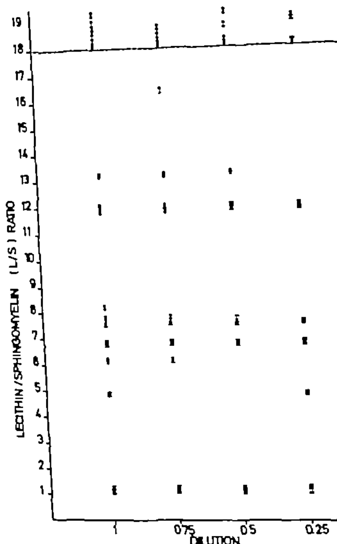


Fig. 2. Amniotic fluid L/S ratio correlated to the bubble stability test. ● Positive. Negative.

and a sphingomyelin concentration of 0.32 µM/100 ml. A similar pattern follows with dilution.

Forty samples of amniotic fluid from 40 mothers were obtained within one week of delivery. The relationship between the BST carried out on undiluted amniotic fluid, their lecithin concentrations and the development of RDS is presented in Fig. 3. All samples with a positive test in undiluted amniotic fluid (30) were from mothers who delivered healthy babies. The lowest lecithin concentration in these samples was 4.56 µM/100 ml. Ten samples had negative BST and eight of these were from mothers whose babies developed RDS. Of these four samples with lecithin concentrations from 1.3–4.27 µM/100 ml were associated with mild RDS and four samples with lecithin concentrations >1.0 µM/100 ml were associated with severe RDS. The remaining two samples with a negative BST were from mothers whose babies were healthy. The lecithin concentrations were 3.07 and 4.04 µM/100 ml respectively.

The relationship between the BST in the undiluted amniotic fluid and the calculated L/S ratio for each sample is presented in Fig. 4. All samples with positive BST when undiluted had a L/S ratio of more than 5.2. Of the eight samples from mothers whose babies developed RDS, seven had a L/S ratio below 4.7 and one with lecithin concentration of 4.27 µM/100 ml had L/S ratio of 6.24. The two samples with a negative BST not

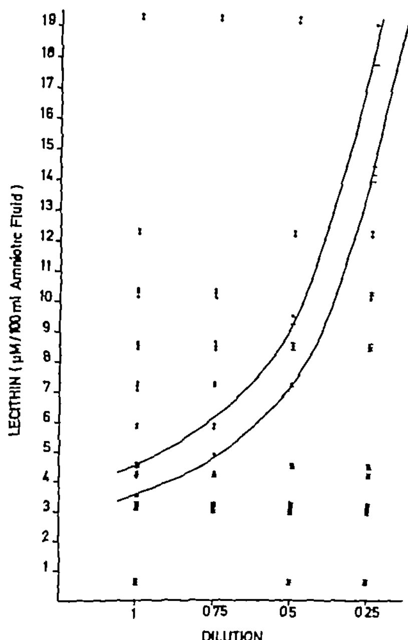


Fig 1 Lecithin concentrations in amniotic fluid correlated to the bubble stability test ● Positive – Negative

sample is presented in Fig. 1 Thirty-three samples containing more than 4 6 µM/100 ml of lecithin were all positive when tested undiluted with the BST Eighteen samples with lecithin concentrations less than 3 6 µM/100 ml were negative Nine samples had lecithin concentrations between 3 6 and 4 6 µM/100 ml Of these five showed a positive BST in the undiluted tube and four showed a negative BST The two lines in Fig. 1 begin at the undiluted samples of amniotic fluid and show the lecithin concentrations above which all tests were positive and below which all tests were negative The lines are then continued through the corresponding areas of concentration of lecithin for each dilution. The range of lecithin concentrations for the undiluted samples where the test can either be positive or negative is shown to be 3 6–4 6 µM/100 ml A similar pattern follows for each dilution however the correlation is somewhat less clear It may be seen that the highest amniotic fluid lecithin concentration with an intermediate BST is 11 6 µM/100 ml

Results of the BST for the same samples plotted against the calculated L/S ratio are presented in Fig 2 All samples with a L/S ratio less than 5.8 showed a negative BST when undiluted Of 45 samples with a L/S ratio of 5.8 or more eight were negative and the rest positive when undiluted The highest L/S ratio with a negative BST was 14 1 with a lecithin concentration of 4 5? µM/100 ml

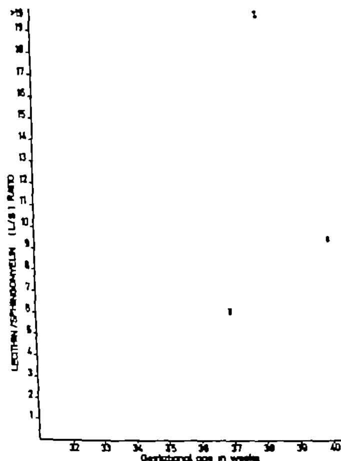


Fig. 4. Relationship between the L/S ratio and the bubble stability test carried out on undiluted amniotic fluid, with fetal outcome and gestational age. O Positive BST with healthy infant. Δ, Negative BST with healthy infant. ▲, Negative BST where infant developed respiratory distress.

lecithin concentration was found to be 4.56 μM/100 ml, which is in close agreement with Bhagwanani's findings (1) that lecithin concentrations over 3.5 mg/100 ml indicates sufficient fetal lung maturity for respiration to be normal. In our series of 10 negative tests, four infants developed severe RDS, four were mildly distressed and two were healthy which was more in agreement with the findings reported by Clements.

Altogether these findings make interpretation of the BST alone difficult. We agree that positive BST indicates mature fetal lungs. Although none of our babies with an intermediate BST developed RDS this may occur since we have had positive test in the undiluted tube with a lecithin concentration of 3.6 μM/100 ml only. Our findings confirm that a negative BST carries high risk of RDS (8 out of 10). However it has two limitations. Firstly it gives no quantitative measure of the lecithin concentration, thereby limiting its prognostic value. Babies delivered from mothers whose amniotic fluid lecithin concentration is in the range of 3.0 to 4.6 μM/100 ml may have only mild distress or be healthy. At levels below 1.0 μM/100 ml, the prognosis for the baby however is serious. Secondly it is our experience that it is rare to find amniotic fluid lecithin levels >4.6 μM/100 ml prior to 35 weeks gestation, thereby limiting the value of the BST at earlier gestations. Our findings of a high probability that respiration would be normal with intermediate results and also that negative tests may give false results, makes it necessary to have some other means of determining lecithin concentrations in these instances as well as before 34–35 weeks of gestation.

The significance of sphingomyelin in amniotic fluid is less clear since it tends to show some individual variation and little variation with gestational age. One may speculate that sphingomyelin reflects the intra-uterine quantity of amniotic fluid and be

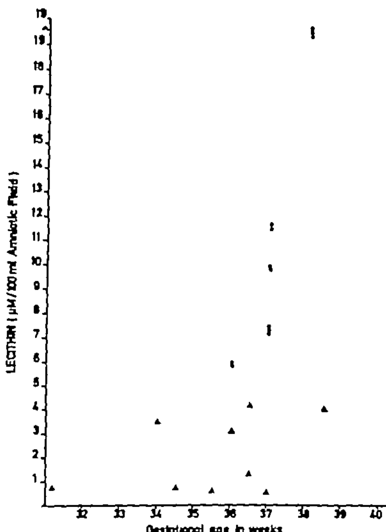


Fig 3 Relationship between the lecithin concentration and the bubble stability test carried out on undiluted amniotic fluid, with fetal outcome and gestational age O Positive BST with healthy infant Δ Negative BST with healthy infant ▲ Negative BST where infant developed respiratory distress.

associated with RDS showed L/S ratios of 7 5 and 5 3 respectively L/S ratios between 2 1 and 4 7 were associated with mild RDS

The earliest sample with a positive BST in undiluted amniotic fluid was obtained at 35 weeks gestation. The lecithin concentration in this case was 5 64 μM/100 ml and the L/S ratio was 5 3

DISCUSSION

The test developed by Clements is based on the following observation When amniotic fluid is mixed in equal parts with 95% ethanol and shaken a stable foam is generated only in the presence of phospholipids having two saturated fatty acids We have previously shown that the lecithin is the major phospholipid component of amniotic fluid (5) and that it starts to rise sharply from 34–35 weeks gestation when pulmonary maturation proceeds normally In the series of experiments presented in Fig 1 and Fig 3 a positive BST in the first three tubes was associated with lecithin concentrations >11 7 μM/100 ml In samples with intermediate BST results the lecithin concentrations were from 3 6–11 6 μM/100 ml Negative BST results could be found with concentrations in the range of 3 6–4 6 μM/100 ml and the BST was always negative with concentrations less than 3 6 μM/100 ml. Fig 2 shows that variations in the sphingomyelin level altering the L/S ratio considerably correlated poorly with the BST This shows that bubble stability is chiefly dependent upon the amount of lecithin present

Clements considers a positive BST in undiluted 0 75 and 0 5 dilutions of amniotic fluid to indicate safe delivery with normal respiration in the newborn and intermediate results to carry a high probability of RDS This risk is considered very high in cases with negative tests In the series of experiments presented in Fig 3 and Fig 4 intermediate results were associated with normal respiration in all cases This was unexpected but the lowest

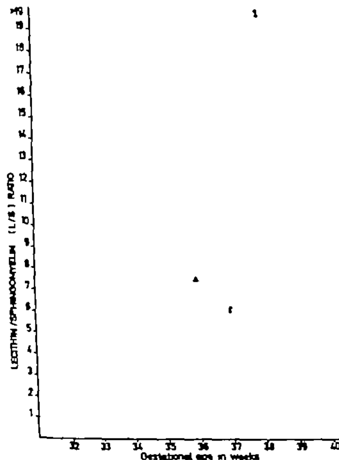


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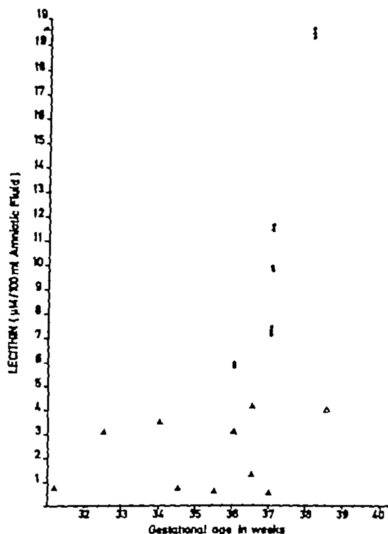


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LECITHIN-BOUND PALMITIC ACID AND LECITHIN/SPHINGOMYELIN RATIO OF AMNIOTIC FLUID IN RELATION TO FETAL MATURITY

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Abstract The proportion of lecithin-bound palmitic acid and the lecithin/sphingomyelin-ratio were determined in human amniotic fluid during the third trimester. All the cases studied terminated in the birth of healthy children. The proportion of palmitic acid in the lecithins increased with advancing gestational age until 39–40 weeks, when it flattened off and then tended to decline slightly. The lecithin/sphingomyelin-ratio also increased with advancing gestational age but within each week there was considerable variance. Complicated pregnancies did not differ from normal ones. The results indicate that determination of the proportion of lecithin-bound palmitic acid in amniotic fluid might be an additional variable of clinical value in estimating foetal maturity.

Certain complications of pregnancy such as Rh-immunization, diabetes mellitus toxicosis and placental insufficiency often indicate induction of labour. In such cases knowledge of the maturity of the foetus is desirable but the methods available for this purpose are still unsatisfactory.

Several methods have been devised and tried in determination of certain components of the amniotic fluid such as the concentration of creatinine which has been found to increase with advancing gestational age (5 6, 7 9 10). The concentration of uric acid (5) and the proportion of orange staining cells (2 6 9 1 15) have also proved to increase with gestational age. The lecithin/sphingomyelin-ratio has recently been studied as an index not only of pulmonary maturity but also of gestational age (3 7 8 13 14 16).

We compared the amniotic fluid phospholipids in the 2nd trimester with those at full term and also analysed the fatty acid composition of the phosphatidylethanolamines and the lecithins (1). Not only the lecithin/sphingomyelin-ratio, but also the proportion of palmitic acid in the lecithins were found to be significantly higher in the full-term group. This paper concerns the phospholipids of amniotic fluid from pregnancies between the 28th and 43rd week terminating in the birth of healthy children. Determinations were made of the proportion of palmitic acid in the lecithins as well as of the lecithin/sphingomyelin-ratio.

MATERIAL AND METHODS

92 samples of amniotic fluid obtained from 89 pregnancies that terminated in the birth of healthy children were analysed. No case in which the infant developed Idiopathic Respiratory Distress Syndrome (IRDS) or Hyaline Membrane Disease (HMD) was included. Thirty of the pregnancies were complicated. The complications were Rh-immunization in 11 cases, toxicosis (B P>140/90, oedema and/or proteinuria) in eight, suspect placental insufficiency in three, abruptio placentae in five placenta praevia in two and diabetes mellitus in one. The maturity of the infants at parturition was estimated by paediatricians according to the criteria of Koenigsberger (*11*) and Usher et al (*17*).

The samples of amniotic fluid were obtained at amnioscopy 30 min to 5 hours before parturition. The pole of the amniotic sac was visualized and punctured, after which about 40 ml of amniotic fluid was collected via the cannula directly into centrifuge tube. With this procedure it was possible to avoid contamination of the sample with vaginal secretion and blood. In cases of Caesarean section the samples were obtained by direct puncture of the uterus. In two cases of Rh-immunization repeated transabdominal amniocenteses were done. The samples were prepared in the way described previously. Immediately centrifuged at 1000 g for 15 min after which the supernatant were decanted and stored at 20°C until analysed (*4*). Storage under these conditions did not affect the analytical result (*4*).

useful in instances when these quantities are either abnormally large or abnormally low In such cases it has been our clinical impression that the L/S ratio gives a better indication of fetal pulmonary maturity than the lecithin concentration alone

The discrepancies in the lecithin concentrations and the L/S ratios associated with RDS in this series of experiments compared with our previous results (5) can be explained by the effect which centrifugation prior to lipid extraction has on amniotic fluid phospholipid recovery (6)

CONCLUSIONS

The results of this study presented in the first series of experiments show that there is close correlation between the amniotic fluid lecithin concentrations and the generation of stable bubbles in undiluted amniotic fluid carried out by the bubble stability test This correlation decreases with dilution of the amniotic fluid. The L/S ratio and the sphingomyelin content correlated less well with BST showing that bubble stability is dependent upon the quantity of lecithin present

The second series of experiments show that a positive BST in undiluted amniotic fluid 0 75 and 0 5 dilutions was associated with a high lecithin content and mature fetal lungs Intermediate results carried a low risk of RDS and a negative BST carried a high risk of RDS The low lecithin concentrations in amniotic fluid prior to 34–35 weeks gestation suggest that the BST is only of limited value at such gestational ages

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REFERENCES

1 Bhagwanani S. G et al., Prediction of neonatal respiratory distress by extraction of amniotic fluid lecithin. Lancet *43* 77 1972.
2 Bhagwanani S G et al Bubble stability test compared with lecithin assay in prediction of respiratory distress syndrome. Br Med J *1* 697–700 March 24 1973
3 Clements, J A. et al Assessment of the risk of the respiratory distress syndrome by a rapid test for surfactant in amniotic fluid. N Engl J Med 286. 1077 1972.
4 Gluck L. et al Procedure for the intrauterine assessment of pilmonary maturity by the phospholipids in amniotic fluid. Newsletter Univ of Cal. San Diego, Aug. 1 1971
5 Lindback T et al., Phospholipids in amniotic fluid. Acta Obstet Gynecol Scand *53* 219 1974.
6. Lindback T & Frantz, T Effect of centrifugation on amniotic fluid phospholipid recovery Acta Obstet Gynecol Scand submitted for publication
7 Nelson G H Amniotic fluid phospholipid patterns in normal and abnormal pregnancies. Am J Obstet Gynecol *105* 1072 1969
8. Wagstaff T I & Bromham, D R. A comparison between the lecithin/sphingomyelin ratio and the shake test for estimation of surfactant in amniotic fluid. J Obstet Gynaecol Br Comm *80* 41 –417 1973

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Table I. *Individual values for two cases of Rh-immunization, where serial samples were obtained*

Case	Weeks of gestation	Lec./sph. (moles/mole)	Palmitic acid in lecithin (mole %)
	31	2.7	64.6
	35	4.4	83.1
	38	7.7	90.1
	34	3.9	6[illegible].8
	36	5.6	68.5

DISCUSSION

The proportion of palmitic acid in the amniotic fluid lecithins increased with advancing gestational age until 39–40 weeks after which it tended to decrease. The lecithin/sphingomyelin-ratio also increased continuously but with a wider range of variation within each week than that of the proportion of palmitic acid.

An abrupt rise in the lecithin/sphingomyelin-ratio in the 35th week of pregnancy has been reported (8, 13). Schulman et al. (14), who analysed 68 cases, found the lecithin/sphingomyelin-ratios to increase with advancing gestational age as judged from the mean lecithin/sphingomyelin-ratios for the age groups studied. Remarkably enough, in some cases analyses of serial samples showed no correlation with gestational age. Like Donald et al. (3) we found a progressive increase in the lecithin/sphingomyelin-ratio with gestational age but a considerable range of variation within each week throughout the third trimester. In the two cases of Rh-immunization where serial samples were analysed we found the lecithin/sphingomyelin-ratio to increase with advancing gestational age which is in agreement with observations by Gerbie et al. (7).

According to Gluck et al. (8) the lecithin/sphingomyelin-ratio is correlated with gestational age only in normal pregnancies. They also claim that the lecithin/sphingomyelin-ratio increases earlier in cases of hypertonia, placental insufficiency, retroplacental bleeding and premature rupture of the membranes but more slowly in some diabetic pregnancies. We found no difference between the ratios in complicated and normal pregnancies. This is in line with the finding by Donald et al. (3) that complicating toxaemia and diabetes had no effect on the ratio in question.

In contrast with the above investigations we have performed our phospholipid analyses without preceding acetone precipitation and the lecithin and sphingomyelin fractions were chemically quantitated by determination of phosphorus. With the method used by the others a subfraction of the total phospholipids is analysed and the lecithin/sphingomyelin-ratio is measured densitometrically.

The increase in the proportion of palmitic acid in the amniotic fluid lecithins was correlated more closely with gestational age than the lecithin/sphingomyelin-ratio (47% and 31% respectively of the total variation was explained by the regression). Like the lecithin/sphingomyelin-ratio it was not influenced by complications of pregnancy. However, the relative amount of lecithin-bound palmitic acid tended to decrease after the 40th week of gestation. At present an interpretation of this finding is precluded by the limitations of our knowledge on the origin and metabolism of the amniotic fluid lecithins. The results agree with our previous study in which the proportion of palmitic acid in the lecithins was found to be significantly larger at full term than in the 2nd trimester (1). But we have also found a low proportion of palmitic acid in those cases where the children developed IRDS or HMD (4). Thus, but for this exception, determination of the proportion of palmitic acid in the amniotic fluid lecithins might be an additional variable of clinical value in estimating foetal maturity.

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REFERENCES

1. Arvidson, G., Ekelund, H. & Åstedt, B.: Phospholipid composition of human amniotic fluid during gestation and at term. Acta Obstet Gynecol Scand 51: 71, 1972.
2. Barnett, H. & Nevin, M.: The value of the Nile Blue Test in estimating fetal maturity in normal and complicated pregnancies. J Obstet Gynaec Br Comm 77: 151, 1970.

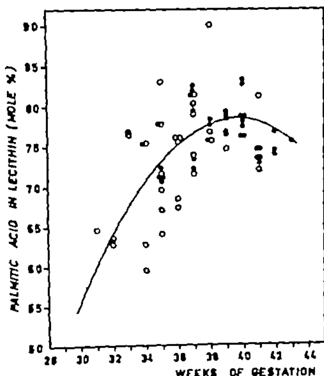


Fig. 1. Relation between palmitic acid content of amniotic fluid lecithin and gestational age in the third trimester of pregnancy. The curve fitted to the data is represented by the equation $y = -309.1 + 19.53x - 0.246x^2$. 19.53+0($p<0.001$) 0.246+0($p<0.001$) S.D. = 4.9. ● = normal pregnancies. O = complicated pregnancies.

Analytical

Each sample was concentrated *in vacuo* at room temperature to a volume of 4–5 ml after which 75 ml of chloroform/methanol 2:1 (v/v) was added, yielding a homogenous solution. The mixture was filtered into separating funnels. After addition of 0.4 volumes of 0.9% NaCl the funnels were left over-night. The phospholipids in the chloroform-phase thus obtained were analysed by methods previously described in detail (1). With the aid of thin-layer chromatography on silica gel the phospholipids were separated into six fractions. The lecithin and sphingomyelin fractions were quantitatively eluted from the silica gel and their phosphorus content was determined spectrophotometrically. The fatty acid composition of the lecithins was determined by gas-liquid chromatography after transesterification in methanol/H_2SO_4.

Statistical methods

Relations between various factors and gestational age were studied with polynomial regression and linear regression analysis.

RESULTS

The proportion of palmitic acid in the lecithin increased with advancing gestational age until 39–40 weeks when it flattened off and then tended to decline slightly. Polynomial regression (2nd degree) revealed significant linear as well as nonlinear components. The values found for complicated pregnancies did not deviate from those found for normals. (Fig. 1.) Even in the two cases of Rh-immunization where serial samples were analysed the proportion of palmitic acid in the lecithins increased with advancing gestational age (Table I). Also the lecithin/sphingomyelin-ratio increased with advancing gestational age ($r=0.56$, $p<0.001$) but within each week there was a considerable variance. Polynomial regression (2nd degree) did not reveal nonlinearity ($p>0.70$). The values found for complicated pregnancies did not differ from those found for the normals (Fig. 2). Also in the two complicated cases with serial samples the ratio increased with advancing gestational age (Table I).

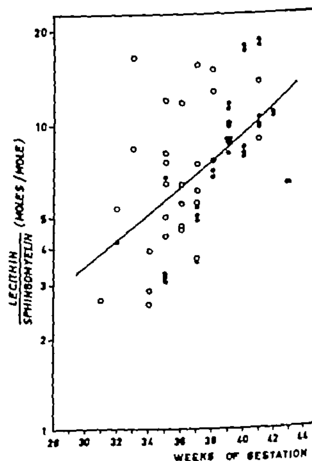


Fig. 2. Relation between lecithin/sphingomyelin ratio in amniotic fluid and gestational age in the third trimester of pregnancy. The curve fitted to the data is represented by the equation log $y = -0.747 + 0.0429x$. 0.0429+0 ($p<0.001$) S.D. = 0.70. r = 0.56. ● = normal pregnancies. O = complicated pregnancies.

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The proportion of palmitic acid in the amniotic fluid lecithins increased with advancing gestational age until 39–40 weeks after which it tended to decrease. The lecithin/sphingomyelin-ratio also increased continuously but with a wider range of variation within each week than that of the proportion of palmitic acid.

An abrupt rise in the lecithin/sphingomyelin-ratio in the 35th week of pregnancy has been reported (8, 13). Schulman et al. (14), who analysed 88 cases, found the lecithin/sphingomyelin-ratios to increase with advancing gestational age as judged from the mean lecithin/sphingomyelin-ratios for the age groups studied. Remarkably enough, in some cases analyses of serial samples showed no correlation with gestational age. Like Donald et al. (3) we found a progressive increase in the lecithin/sphingomyelin-ratio with gestational age but a considerable range of variation within each week throughout the third trimester. In the two cases of Rh-immunization where serial samples were analysed we found the lecithin/sphingomyelin-ratio to increase with advancing gestational age, which is in agreement with observations by Gerbie et al. (7).

According to Gluck et al. (8) the lecithin/sphingomyelin-ratio is correlated with gestational age only in normal pregnancies. They also claim that the lecithin/sphingomyelin-ratio increases earlier in cases of hypertonia, placental insufficiency, retroplacental bleeding and premature rupture of the membranes but more slowly in some diabetic pregnancies. We found no difference between the ratios in complicated and normal pregnancies. This is in line with the finding by Donald et al. (3) that complicating toxaemia and diabetes had no effect on the ratio in question.

In contrast with the above investigations we have performed our phospholipid analyses without preceding acetone precipitation and the lecithin and sphingomyelin fractions were chemically quantitated by determination of phosphorus. With the method used by the others a subfraction of the total phospholipids is analysed and the lecithin/sphingomyelin-ratio is measured densitometrically.

The increase in the proportion of palmitic acid in the amniotic fluid lecithins was correlated more closely with gestational age than the lecithin/sphingomyelin-ratio (47% and 31% respectively of the total variation was explained by the regression). Like the lecithin/sphingomyelin-ratio, it was not influenced by complications of pregnancy. However, the relative amount of lecithin-bound palmitic acid tended to decrease after the 40th week of gestation. At present an interpretation of this finding is precluded by the limitations of our knowledge on the origin and metabolism of the amniotic fluid lecithins. The results agree with our previous study (1) in which the proportion of palmitic acid in the lecithins was found to be significantly larger at full term than in the 2nd trimester (1). But we have also found a low proportion of palmitic acid in those cases where the children developed IRDS or HMD (4). Thus but for this exception, determination of the proportion of palmitic acid in the amniotic fluid lecithins might be an additional variable of clinical value in estimating foetal maturity.

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REFERENCES

1. Arvidson, G., Ekelund, H. & Åstedt, B. Phospholipid composition of human amniotic fluid during gestation and at term. Acta Obstet Gynecol Scand 51: 71, 1972.
2. Barnett, H. & Nevin, M. The use of the Nile Blue Test in estimating fetal maturity in normal and complicated pregnancies. J Obstet Gynec Br Comm 77: 151, 1970.

3 Donald, I R Freeman R K Goebelsmann V Chan W H & Nakamura R M Clinical experience with the amniotic fluid lecithin/sphingomyelin ratio Am J Obstet Gynecol 115 547 1973
4 Ekelund, L, Arvidson G & Åstedt, B., Amniotic fluid lecithin and its fatty acid composition In respiratory distress syndrome J Obstet Gynecol Brit Comm (in press)
5 Enlander D Amniotic fluid indicators of fetal maturity Obstet Gynecol 40 605 1972
6. Gariepy G Amireault F & Barry Y Détermination de la maturité foetale par l étude cytologique et biochimique du liquide de amniotique L union Medicale Du Canada 102 51 1973
7 Gerbie M V Gerbie A B & Boehm J Diagnosis of fetal maturity by amniotic fluid phospholipids. Am J Obstet Gynecol 114 1078 1972
8 Gluck L. & Kulovich M Lecithin/sphingomyelin ratios in amniotic fluid in normal and abnormal pregnancy Am J Obstet Gynecol 115 539 1973
9 Henneman C. E. Anderson G V Tejavej A Gross, H A & Helman V L. Fetal maturation and amniotic fluid. Am J Obstet Gynecol 108 302, 1970
10 Jakobovits A Farkas M & Paukovich I Cytologic patterns and creatinine concentration of the amniotic fluid. Acta Cytol 16 381 1972
11 Koenigsberger M Judgement of fetal age I Neurologic evaluation Pediat Clin N Amer 13 823 1966
12 Magnin P Lansac, J Lieux J -M Sauzay P & Brezlat J P Cytologie du liquide amniotique et maturité foetale Rev Franc Gynéc 67 699 1972.
13 Mercus, J M W M Kock, H C L V Mercus, F W H M Verhoeven A G J & Beyaens, A J M M Evaluation of the amniotic fluid test and its relationship to the respiratory distress syndrome. Am J Obstet Gynecol 115 839 1973
14 Schulman J D Queenan J T Scarpelli E M Church E & Auld P A M Lecithin-sphingomyelin ratios in amniotic fluid Obstet Gynecol 5 697 1972
15 Sharma S D & Trussell R R The value of amniotic fluid examination in the assessment of fetal maturity J Obstet Gynecol Br Comm 77 215 1970.
16 Spellacy W N & Buhi W C Amniotic fluid lecithin/sphingomyelin ratio as an index of fetal maturity Obstet Gynecol 39 852, 1972.
17 Usher R McLean F & Scott K E Judgement of fetal age II Clinical significance of gestational age and an objective method for its assessment. Pediat Clin N Amer 13 835 1966

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COMPARATIVE ASSAY OF HCG HCT AND HCS IN MOLAR PREGNANCY

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Abstract The authors studied the secretion patterns of three chorionic polypeptide hormones (HCG, HCT and HCS) and the clinical usefulness of the assay of these hormones in patient with hydatidiform mole, by means of systematic serial estimation, in comparison with the secretion patterns in normal pregnancy The serum levels of HCG and HCT in molar pregnancy were usually higher than in normal pregnancy but HCS levels were below the lower limit of normal pregnancy of the same gestational age. This dissociation between the chorionic glycoprotein hormones (HCG and HCT) and the simple protein hormone (HCS) thought to be one of the characteristic findings in molar pregnancy Although the amounts of HCG and HCT did not change 6 hours after the evacuation of hydatidiform mole the concentration of HCS decreased rapidly and 60 min later it became undetectable Thus, these polypeptide hormones disappeared in the order HCS HCT and HCG after the evacuation of mole

It is well known that human chorionic somatomammotropin (HCS or human placental lactogen HPL) (1 13) and human chorionic thyrotropin (HCT or human chorionic thyroid stimulating hormone HCTSH) (1 8 9 10 27) well as human chorionic gonadotropin (HCG) are secreted from the placenta

However although the secretory behavior of HCG in molar pregnancy has been investigated the secretory behavior of HCT or HCS or the secretory relationship between these three polypeptide hormones ha not been determined in detail

Accordingly w have compared the results of assay of these three hormones in molar pregnancy t those f om normal pregnancy in order to understand the secretory behavior of these hormones patient with hydatidiform mole and to learn whether the simultaneous assay of them is useful in clinical field

MATERIALS AND METHODS

Clinical subject

10 patients in hospital with diagnosis of hydatidiform mole and 30 patients in the early stage of normal pregnancy (8–16th gestation weeks) were used as the study and control groups

Blood sampling

Blood samples were collected the early morning before treatment for hydatidiform mole and 30 min 60 min, 6 1–, 18, 24 and 48 hours after the evacuation of the mole and thereafter blood samples were collected every eek

Immediately after collection, the blood was centrifuged, and the isolated serum w kept at −70°C

Radio-immunoassay

HCG HCT and HCS were determined at the same time by radioimmunoassay ith the double-antibody method (19)

Antigens

The *HCT* used in the assay was highly purified preparation with biological activity of 125 mU/mg (27 29).

In the assay of HCG HCG with biological activity of 15000 IU/mg which was extracted from urine (2) and placenta (3 17 28) was used Purified HCS was used in the assay of HCS (18, 20).

Antisera

Antisera against these antigens were prepared in guinea pigs.

The purification of HCT-antiserum was performed by absorption with 5000 IU HCG/ml and 90 mg human serum albumin 1/ml antiserum. The purification of HCG-antiserum and HCS-antiserum was performed by absorption with human serum albumin 90 mg/ml.

The second antibody rabbit antiserum was prepared by the injection of guinea pig gamma globulin in rabbits. Serum from normal guinea pigs was used as the carrier protein in order to prevent error in the assay system.

Table I HCG HCT and HCS in n rmal pregnancy*

HCG range (IU/ml)	HCT range (μg/ml)	HCS range (μg/ml)
8–300	2.5–18 0	0 02–0 9

The polypeptide hormones were measured in 30 cases of normal pregnancy from the 8th week to the 16th week of gestation.

Labelling of antigens

The antigens were labelled with I (New England Nuclear Corp. USA) according to Greenwood & Hunter (6) with additional modifications (.5 76 79)

The purification of labelled hormones was performed by gel filtration on a Sephadex G 75 column (10×300 mm)

The specific activity of the hormones labelled was nearly 700–.50 μCi/μg.

Standa d preparations

Standard preparations employed in the assay of these hormones were Second International Standard HCG (National Institute for Medical Research Mill Hill London) and HCT and HCS purified by the authors

The unit of HCG was expressed in the radio-immunoassay as IU to nd IS HCG However the units of HCT and HCS were expressed as μg of authors HCT and HCS because there are no internationally standardized units

In these assay systems the sensitivity to HCG was 0.5 mU/ml to HCT 0 1 μg/ml and to HCS 0 005 μg/ml

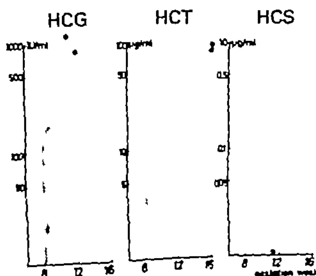


Fig 1 HCG HCT and HCS in molar pregnancy (8–16th week of gestation) Shaded area denotes normal range of these hormones Units for HCG HCT and HCS were expressed on logarithmic scale

RESULTS

Blood HCG HCT and HCS in normal early pregnancy

The normal level of HCG assayed in our cases ranged from 8 to 300 IU/ml reached the peak at the end of the 10th week and gradually decreased thereafter

HCT became detectable at the end of the 8th week of pregnancy and its normal level ranged from 2.5 to 18 0 μg/ml throughout the early stage of pregnancy

HCS also became detectable at the end of the 8th week Its concentration ranged from 0.04 to 0.3 μg/ml at the end of the 12th week and reached about 1 0 μg/ml at the end of the 16th week after an abrupt rise (Table I Fig 1)

Blood HCG HCT and HCS in m lar pregnancy

The blood levels of HCG and HCT assayed in molar pregnancy ranged from 250 to 1700 IU/ml and from 40 to 100 μg/ml respectively which are distinctly high levels compared with those in normal pregnancy The HCS level ranged from 0 012 to 0 04 μg/ml and was abnormally low (Fig 1)

Changes in blood HCG HCT and HCS after molar delivery

There was no change in blood HCG and HCT levels 6 hours after molar delivery The HCS level decreased rapidly and reached about 0 01 μg/ml after 6 hours and became undetectable thereafter

HCT became undetectable nearly 5 days after molar delivery but about 3 weeks were necessary before the level of HCG became to be at the pituitary LH level (80 IU/l) (Fig 2)

DISCUSSION

It is well known that a large amount of HCG is excreted in the urine in patients with hydatidiform mole compared with the level in normal pregnancy and blood HCG behaves similarly (16) Consequently sensitive assay f HCG is important in evaluation of the effect of treatment in patients with hydatidiform mole and this assay is now routine in the management of chorionic tumor

On the other hand the association of hyperthyroidi m with molar pregnancy has been re

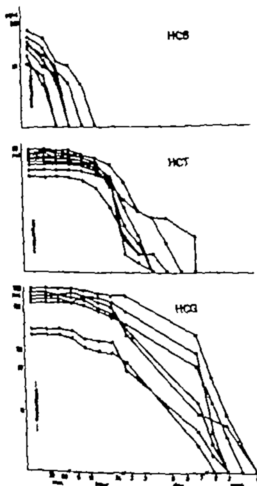


Fig. 2. HCG, HCT and HCS after the evacuation of hydatidiform mole. Unit for HCG HCT and HCS were expressed on logarithmic scale

ported by Kupperman (15), Odell et al (21 22) and Kristoffersen et al (14). It has been suggested that a specific thyroid stimulating substance may be produced from molar ti sue because the hyper thyroidism disappears in patient after molar de livery

Recently Hershman t al (9 11) have proved the existence of thyroid stimulating ubstance that is immunologically nd biologically different from pituitary TSH or LATS in the blood and molar tissue from patient w th hydatidiform mole associated w th hyperthyroidism

The authors (29) ha confirmed the evidence that the blood le ls of pituitary TSH in hydatidiform mole are the same as the levels in normal pregnancy but that the blood level of HCT is abnormally high and have reported that an abnormally large amount of HCT is excreted from molar tissue

Unlike the glycoproteins HCG and HCT HCS is a simple protein hormone The excretion pat tern of this hormone has been studied by many researchers mainly in the evaluation of placental function However the secretory pattern of these polypeptide hormones and their properties have not been nalysed i detail in cases of hydatidi form mole

For this reason we have studied the secretory pattern of three hormones HCG HCT and HCS by the systematic simultaneous assay in hydatidi form mole using a highly sensitive radio-immunoassay

In order to assay the blood level of polypeptide hormones by the immunological method it is first necessary to establish the specific antigen-antibody system of the hormone to be assayed For this purpose we have extracted and purified HCG (HCLH) HCT and HCS from the placenta and prepared antisera against the respective hormones

According to our study using the specific antigen-antibody systems of the respective hormones evidence was obtained that the blood levels of HCG and HCT were abnormally high and that of HCS was abnormally low in hydatidiform mole as compared with their levels in normal pregnancy and the weekly increasing pattern of HCS in nor mal pregnancy was not observed

The low level of HCS has been reported by Frantz et al (5) Samaan et al (23) Saxena et al (24) and Grumbach et al (7) and their results were similar to the authors

That is to say according to the quantitati e analysis of polypeptide hormones molar preg nancy seems to be characterized by "high chorionic glycoprotein hormones and low chorionic simple protein hormone compared with normal pregnancy

Consequently the difference seen between the secretion patterns of HCG HCT and HCS is thought to be one of the characteristic findings in hydatidiform mole

From the results thi difference revealed by the ystematic simultaneous assay of polypeptide hormones is regarded a one of the reliable indica tions in the diagnosis nd treatment of chorionic tumors However it is still not known whether the

assay of polypeptide hormones is an indicator of the adequency of treatment in chorionic tumors

As already described blood HCS became undetectable within from 60 min to 6 hours after complete evacuation of the hydatidiform mole The blood levels of HCG and HCT did not change within 6 hours after the operation It required about 3 weeks for the HCG level to reach the level of pituitary LH It required about 5 days for HCT to become undetectable On the other hand in cases in which hydatidiform mole was evacuated incompletely HCG HCT and HCS continued to be detectable after the operation (4)

In short the time required for the disappearance of these polypeptide hormones after molar evacuation was longest for HCG followed by HCT and the shortest for HCS

Accordingly in the treatment of chorionic tumors it seems that assay of the HCG level is of course necessary that the systematic simultaneous assay of HCT and HCS is useful as an indicator of the completeness of treatment and that the assay of HCS is a more sensitive indicator of the effect of treatment after molar delivery

REFERENCES

1 Akasu, F Kawahara S Ohki, H Haraoo M & Tejima, Y Thyroid stimulating hormone extracted from human placenta. Endocrinol Japon 2 297 1955
2. Ashitaka Y Tokura, Y Tane M Mochizuki M & Tojo S Studies on the biochemical properties of highly purified human chorionic gonadotropin. Endocrinology 87 233 1970
3 Ashitaka, Y Mochizuki, M & Tojo S Purification and properties of chorionic gonadotropin from the trophoblastic tissue of hydatidiform mole Endocrinology 90 609 1972
4 Chough S Y Study on the systematic use of serial estimations of human placental lactogen (hPL) and human chorionic gonadotropin (hCG) for the management of trophoblastic neoplasia Endocrinol Japon 20, 9 1973
5 Frantz, A G Rabkin, M T & Friesen H Human placental lactogen in choriocarcinoma of the mole. Measurement by radioimmunoassay J Clin Endocrinol 25 1136, 1965
6. Greenwood F C Hunter W M & Glover J S The preparation of 131I labelled human growth hormone of high specific radioactivity Biochem J 89 114 1963
7 Grumbach M M, Kaplan S, Sciarra J J & Burr L. Chorionic growth hormone-prolactin (CGP): secretion disposition, biologic activity in man and postulate function as the "growth hormone" of the second half of pregnancy Am N Y Acad Sci 14 501 1968
8. Hennen, G Pierce J G & Freychet, P Human chorionic thyrotropin, further characterization and study of its secretion during pregnancy J Clin Endocrinol 29 581 1969
9 Hershman J M Higgins, H P & Starnes, W R Differences between thyroid stimulator in hydatidiform mole and human chorionic thyrotropin. Metab Clin Exp 19 735 1970.
10. Hershman J M & Starnes W R. Placental content and characterization of human chorionic thyrotropin J Clin Endocrinol 32 52, 1971
11 Hershman J M & Higgins H P Hydatidiform mole—a cause of clinical hyperthyroidism report of two cases with evidence that the molar tissue secreted a thyroid stimulator N Engl J Med 28 573 1971
12. Ito Y & Higashi K. Studies of the prolactin-like substance in human placenta. Endocrinol Japon 8 279 1961
13 Josimovich J B & MacLaren, J A. Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone Endocrinology 71 209 1962
14 Kristoffersen K & Sharma Jorgensen, F A case of hydatidiform mole with severe preeclampsia and severe disturbances in thyroid function. Acta Obstet Gynecol Scand 49 119 1970
15 Kupperman H S. & Epstein H S In Essentials of Human Reproduction (ed J T Velardo) p. 95 Oxford Univ Press, London and New York 1958.
16. Loraine, J A. & Bell E. T Hormone assays and their clinical application 3rd ed Livingstone Edinburgh, 1966.
17 Mochizuki M Ashitaka Y Tokura, Y & Tojo S Comparative study of the biochemical character of hCGs from trophoblastic tumor and normal pregnancy Folia Endocrinol Japon 41 482, 1968
18 Mochizuki M Morikawa, H Tanaka, Y & Tojo S Biological characteristics of highly purified human placental lactogen (hPL) Excerpta Medica International Congress Series No 256, abstract 37 at the IV International Congress of Endocrinology Washington D C 1972.
19 Morgan C R & Lazarow A Immunoassay of insulin two antibody system (plasma insulin level of normal subdiabetic and diabetic rat) Diabetes 12 115 1963
20. Morikawa, H Mochizuki M & Tojo S Purification of human placental lactogen and its clinical application by single radical immunodiffusion Endocrinol Japon 18 417 1971
21 Odell, W D Wilber J F & Utiger R D. Studies of thyrotropin physiology by means of radioimmunoassay Recent Progr Horm Res 23 47 1967
22 Odell W D Hertz R. Lipsett M B Ross G T & Hammond C B Endocrine aspect of trophoblastic neoplasms. Clin Obstet Gynecol 10 290 1967
23 Samman S Yen S C C. Friesen H & Pearson,

H Serum placental lactogen levels during pregnancy and in trophoblastic disease J Clin Endocrinol 26. 1303 1966.

24. Saxena, B. N. Refetoff, S Emerson, K. Jr & Selenkow H A rapid radioimmunoassay for human placental lactogen. Am J Obstet Gynecol *101* 874 1968.

25. Tojo S. Mochizuki M. Tokura, Y & Mizunawa, T Radioimmunoassay for the chorionic gonadotropin Clin Endocrinol *17* 6, 1969 (in Japanese).

26. Tojo, S Mochizuki, M & Morikawa, H Immunological characteristics of highly purified human placental lactogen (hPL) and its clinical applications by radioimmunoassay Clin Endocrinol *19* 283 1971 (in Japanese)

27. Tojo S Kanazawa, S., Kitagaki, S. & Mochizuki, M Studies on human chorionic TSH-like substance (HCT S) Folia Endocrinol Japon 48 364 1972.

28. Tojo S Mochizuki, M. Ohga, Y Shimura, S., Maruo T & Nishimoto T Studies of purified human chorionic gonadotropins extracted from trophoblastic tissue Folia Endocrinol Japon 48 639 1972.

29. Tojo S., Kanazawa, S Nakamura, A. Kitagaki, S & Mochizuki M Human chorionic TSH (hCTSH, hCT) during normal or molar pregnancy Endocrinol Japon 20 N 5 1973 (in press).

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MIGRATION OF SPERMATOZOA IN CERVICAL MUCUS FROM WOMEN USING COPPER INTRAUTERINE DEVICES

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Abstract It is widely believed that the contraceptive effect of the copper IUD is due to its inhibition of the penetration and motility of the spermatozoa in cervical mucus containing copper This is based on in vitro studies on midcycle cervical mucus incubated with copper wire. This could not be corroborated by the present investigation of cervical mucus from women using a copper IUD Cervical mucus collected from these women at the time of ovulation showed no reduction in motility or penetration of the spermatozoa

One of the latest contributions to the contraceptive armamentarium is the copper intrauterine device (IUD) The mode of action of this device is not properly understood Chang & Tatum (1) believe that copper interferes with the implantation of the blastocyst It has also been claimed that copper may be toxic to both the spermatozoa and the blastocyst (2 3 4) It is well known that the use of other IUDs results in an increase in the infiltration of leucocytes into the uterine cavity and the endometrium Moyer (5) found that such infiltration was heavier and the contraceptive effect therefore possibly stronger when the devic was made of copper It is also possible that copper modifies the physicochemical properties of the cervical mucus (4)

Hagenfeldt (6) found an increased concentration of copper in the cervical mucus from patients using coppe IUD The finding might help to explain why the presence of copper has been found to suppress the motil ty of spermatozoa in cervical mucus in vitro Loewit (2) found that if a piece of copper wire was placed in a fresh human ejaculate in itro it uppressed the motility of the spermatozoa. Such experiments gave the same results in rat and dog perm (7). Kesserü et al (8) and Elstein & Ferre (9) showed that when a copper wire is placed in the midcycle cervical mucus *in itro* it suppressed the motility of added spermatozoa The midcycle mucus was taken from healthy women who were not using a copper IUD The purpose of the present investigation was to find out whether the cervical mucus in women using a copper IUD has the ability to reduce the penetration and motility of spermatozoa

MATERIAL AND METHODS

Spermatozoal penetration was assessed using Kremer technique (10). At the time of ovulation cervical mucus was collected from 11 women who had been using copper T for at least 6 months. The mucus was afterwards sucked up in 60 mm long capillary tubes. Corresponding mucus from 12 fertile women not using an IUD (or any other contraceptive) served as control material All the mucus samples were kept at −70°C until analysed. The sperm used for the experiment was obtained from donor with normal spermiogram. Sperm migration was afterwards studied simultaneously in all 23 specimens kept for 4 hours in moist chamber at 37°C. During these 4 hours the penetration of the spermatozoa and their motility (11) were recorded every hour

RESULTS AND COMMENTS

No significant difference in motility or penetration (Fig. 1) was found between spermatozoa incubated for four hours with cervical mucus from women using a copper T and mucus from the controls

The motility of spermatozoa incubated with cervical mucus from women using a copper IUD was thus not reduced Neither was the penetration of the spermatozoa significantly reduced These *in vitro* tudies thus argue against earlier published *in vitro* findings. What distinguishes this investigation from others is that we studied cervical mucus from women using a copper T In previous inves-

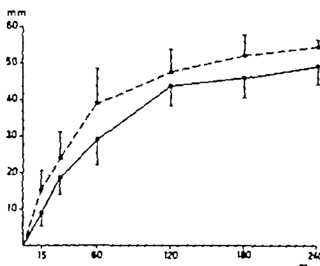


Fig 1 Penetration of spermatozoa after four hours in midcycle cervical mucus from 11 women using a copper T (●——●) and 12 women not using any contraceptive (●- - - -●)

tigations (8 9) use was made of mucus from women not using a copper T This mucus was incubated *in vitro* with a copper wire and spermatozoal penetration was assessed We find such an examination procedure less physiological than methods using mucus from patients with a copper IUD *in situ* The discrepancy between our findings and those on record might perhaps be explained by a difference in the copper concentration in the cervical mucus incubated with a copper wire *in vitro* and that in the mucus taken from women with a copper device in the uterus However Elstein et al (9) found that the copper concentration was the same in both cases But the investigation referred to the total copper content and nothing is known about the form in which the copper occurs in the different types of experiments The contraceptive action of copper should not be sought in decreased penetration or motility of the spermatozoa as a result of an increased concentration of copper in the cervical mucus

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REFERENCES

1 Chang, C C. & Tatum H J Contraception 765 1970
2 Loewit K Immobilization of human spermatoz with iron Basis for a new contraceptive? Contraception 3 219–225 1971
3 MacLeod J Sulfhydryl groups in relation to th metabolism and motility of human spermatozoa. Gen Physiol 34 705–714 1951
4 Oster G K Chemical reactions of the copper i trauterine device Fertil Steril 23 18–23 1972.
5 Moyer D L. Personal communication to I Southam *In* Nobel Symposium No. 15 (ed. I Diczfalusy and U Borell) p 192. Almqvist & Wi sell Stockholm, 1970
6 Hagenfeldt K Intrauterine contraception with th copper T device Contraception 6 37–54 1972.
7 Saito, S Bush, I M & Whitmore W F Effect of certain metals and chelating agents on rat an dog epididymal spermatozoan motility Fertil Ster 18 517–529 1967
8 Kesserü E. & Camacho-Ortega P Influence c metals on *in vitro* sperm migration in the huma cervical mucus. Contraception 6 31– 40, 1972.
9 Elstein M & Ferrer K The effect of a copper releasing intrauterine device on sperm penetratio in human cervical mucus in vitro J Reprod Fert 32 109–111 1973
10 Kremer J A simple sperm penetration test. Intern J Fertil 10 209–215 1965
11 Eliasson R Standards for investigation of huma semen. Andrologie 3 49–64 1971

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RECENT OBSERVATIONS

TUBAL PREGNANCIES IN WOMEN USING PROGESTAGEN-ONLY CONTRACEPTION

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Abstract Nine tubal pregnancies in women using progestagen-only (norethisterone 0.30 mg daily) for contraception are reported. This number is so high in relation to the number of mini-pill users that causal relationship is suspected

The first report on tubal pregnancy in women using progestagen-only for contraception appeared in 1969 (10). Since then eight similar cases have been reported in medical journals (2, 3 4 5 6) making a total of ten. In addition, mention has been made of 13 unpublished cases (3). The progestagens used were chlormadinone 0.5 mg (3 10) ethynodiol diacetate 0.35 mg (4) and norethisterone 0 35 mg (2 5 6).

We can now add nine cases t the list All of these women were taking norethisterone 0.3 mg daily and the duration of mini-pill usage was from 2 to 18 months when they developed the tubal pregnancies The clinical characteristics are pre sented I Table I Three of the patients were treated for inflammatory conditions before the cor rect diagnosis was made A false sense of security on the part of the doctor due to the fact that the subjects were taking hormonal contraception, may have contributed to this delay

The nine cases of tubal pregnancy I women who took mini-pills was a chance observation in four hospitals during less than two years Progestagen-only contraception has been widely used by Norwegian women since the mini-pills were introduced in 1971 Through official sales statistics it is estimated that approximately 15 000 women used the mini-pill in 1973 The question naturally

Table I *N ne omen who had ectopic pr gnancies hile taki g proge tagen-only pills (orethisterone 0.3 mg) for contraception*

ACH Akershus Central Hospital, AACH Aust Agder Central Hospital SH, Stensby Hospital, HH Haukeland Hospital

Patient number	Age	Gravida	Para	Duration of "mini-pill" use	Localization of pregnancy	Hospital
1	22		2	2 months	Left tubal ampulla	ACH
2	38	3	2	4 months	Right tube	HH
3	20	3	1	3 months	Right tube, isthmus	AACH
4	25	2	1	9 months	Left tubal ampulla	AACH
5	25	4	3	Not started	Right tubal ampulla	AACH
6	21	6	2	6 months	Left tube	SH
7	3.	3		9 months	Left tube	ACH
8	27	3	2	18 months	Left tubal ampulla	ACH
9	?7	2	1	12 months	Right tubal ampulla	ACH

arises whether ectopic pregnancies occur more frequently than expected in mini pill users The four hospitals participating in this report cover three well-defined regions in the Eastern South-Eastern and Western parts of Norway We have obtained the sales figures for these three regions and from these we estimate that about 4000 women were using mini-pills during the period covered by this report The failure rates of norethisterone only contraception vary between 1.5 (8) and 6 0 (9) per 100 woman years More than half of the pregnancies have been interpreted as patient failures A failure rate of 4 would result in 320 pregnancies when 4000 women use the mini-pill for 2 years Using a previous estimate of one tubal pregnancy per 130 total pregnancies for the Oslo region (1) we may expect 2.5 ectopic pregnancies among mini-pill users in the three regions during 2 years while we have in fact observed nine Furthermore this is a minimum number firstly since the collection of the case material was retrospective and unsystematic, and secondly since other hospitals in the three regions may have treated similar cases Bonnar (2) found an unusually high incidence of ectopic pregnancies among mini-pill users and a similar mention was made by Fonder & Vetter (3) This supports our assumption that ectopic pregnancy does occur relatively frequently in mini-pill users One possible explanation for this is altered tubal motility In vitro studies have shown that 19-norsteroids diminish the intensity and frequency of peristaltic tubal movements (7). Another possibility is that the mini pill prevents intrauterine but not extrauterine pregnancies This hypothesis is difficult to prove It is impossible to compare the observed number of tubal pregnancies among mini pill users with the expected number in a similar group of women not practising contraception because of the anovulatory cycles in the former The cervical mucus is considered an important factor in mini pill contraception. This contention must be questioned if the contraceptive action is against the uterine cavity selectively because a cervix factor should prevent intrauterine and extrauterine pregnancies equally Several kinds of progestagen-only pills are on sale in Norway No conclusion can be drawn from the fact that all of the nine ectopic pregnancies occurred in women using one particular preparation which also dominates in the sales statistics

REFERENCES

1 Bergsjø P T norske Lægeforen 90 382 1970
2. Bonnar J Lancet I 170, 1974
3 Fonder A & Vetter B Bull Fed Soc Gynecol Obstet Lang Franc 22 329 1970
4 Friedrich, F & Golob E. Geburtsh Frauenheilk 33 310 1973
5 Günther J Geburtsh Frauenheilk 34 208, 1974.
6 Huntington K. M Lancet I 360 1974
7 Jakobovits A. Gecse A. Piukovich I Szontagh F & Karády I Int J Fertil 15 36 1970.
8. Larsson-Cohn U.. Acta Endocrinol Suppl. 144 1 1970
9 Whyte J C. & Pooransingh C. S Canad Med Ass J 109 295 1973
10 Zographos, G & Bergier G Bull Fed Soc Gynecol Obstet Lang Franc 22 295 1969

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CASE REPORTS

THORACOPAGUS TWINS

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Abstract. A case of thoracopagus twins delivered vaginally is reported. The diagnosis was made when obstruction to delivery was encountered in second stage of labour The monster was breech presentation After perforation of the cervical spine of the first twin extraction was easily performed and stillborn female thoracopagus was delivered by the vaginal route. The eight as 3 850 grams. A detailed autopsy is described.

Human double monsters are extremely rare, and therefore we will give a report of a case of conjoined twins delivered vaginally

CASE HISTORY

A 29-year-old primigravida was admitted to hospital 6 eeks before estimated term, because the membranes had ruptured 4 hours earlier Pregnancy had been uncomplicated

On suspicion of multiple pregnancy an X-ray was performed (Fig 1), and the diagnosis of twins, double breech, wa made Labour started spontaneously cervical dilatation progressed normally until 9 cm. Because of inertia an oxytocin-drip was started Vaginal examination showed tw feet at the level of the spines.

The patient started to bear dow 14 hours after rupture of the membranes Three feet presented at the vulva 15 minutes later A vaginal examination showed breech at the level of the spines, and its two feet at the vulva A third foot was at the level of the knees of the former but the second breech could not be reached The cervix was fully dilated The third foot was now pushed back into the uterus, but the wo other feet ascended th it. The fetal heart sounds ere good A general anaesthetic was given to the patient and thorough vaginal examination wa performed, revealing that the third foot led to large abnormal mass, and the diagnosis of malformation was made. By gentle traction on the two presenting feet the first breech descended and now huge eventration and omphalocele was seen. An examination of the upper vagina and the uterus then confirmed the diagnosis of conjoined twins of thoracopagus type with eventration and omphalocele.

After further traction the arms of the first twin were released The posterior cervical spine of the first twin was perforated and the cerebrospinal fluid drained through catheter and the rest of the monster was then delivered by traction without difficulty

The fused double monster (Fig. 2) was stillborn the sex female Weight 3850 g length 47 cm A single placenta was removed manually Vaginal and cervical tears were repaired, and so was the episiotomy performed during delivery

The puerperium was uneventful.

NECROPSY

As seen in Fig. 2 the fetus was a true thoracopagus monster with symmetrical fusion, the line of fusion extending from the lower part of sternum to the umbilicus. A large ruptured omphalocele was present with intestinal loops and liver protruding through an infraumbilical defect in the ventral abdominal wall of both twins.

The upper and lower limbs showed no malformations. One of the twins had an incision in the neck (from cervical perforation) In the following description this will be termed twin A, and the second twin, B

The placenta measured .5 cm in diameter and a single umbilical cord containing two arteries and one vein arose near the middle. There was one amniotic sac



Fig 1 X-ray taken during labour

Respiratory system The larynx trachea, bronchi and lungs appeared normal in both cases. There was no communication between the pleural cavities of the fetuses and in each case the diaphragm was complete

Alimentary system The fusion involved the whole thickness of the abdominal wall and along the margin of the abdominal communication there was continuity of parietal peritoneum resulting in a common peritoneal cavity

In both cases the oesophagus and the stomach were normal The two duodenums fused 3 cm from each pyloric ring forming a common jejunum and upper ileum 40 cm from the duodenal fusion the common ileum divided into two branches. One of these entered a normal caecum which continued into a normal colon rectum and anus of twin B Also a well developed fetal type appendix was



Fig 2 True thoracopagus monster with symmetrical fusion from sternum to umbilicus



Fig 3 The excised viscera Not the common pericardial sac (*P*) with set of lungs (*L*) on each side (*M*) is



Fig. 4. Excised and dissected viscera. Not the two oesophagi (*E*), the two stomachs (*S*) and the duodenal fusion (*F*) with gall bladder on each side. (*L*) is the fused liver mass, (*M*) the common jejunum and upper ileum (*D*) the division of the common ileum, (*C*) the normal caecum of Twin B and (*G*) the sac-like dilatation imitating an abnormally developed caecum of twin A. Not the two sets of normally developed kidneys (*K*) and the two normal spleens (*X*).

present. The other branch of the dividing common ileum continued to normal anus of twin A but without the formation of caecum, a colon or a rectum. Midway however was found little sac like dilatation from which arose short fibrous string, gi ing the appearance of an abnormally de eloped caecum and appendix, but no taenia were present and there was no alteration in the mucosal pattern.

Massive fusion w present between the liver of twin A and twin B the fusion being so complete that it was impossible to determine the portion referable to each twin. There was one common hepatic duct which received two cystic ducts from two gall bladders lying on the inferior surface of the liver mass. The common bile duct terminated in a common ampulla of Vater situated in the line of the duodenal fusion.

In each case there was a normally developed pancreas and spleen.

Cardiovascular system. There was a common pericardial sac containing two hearts with widespread fusion in the atrial and the ventricular region. The heart of twin B was the larger measuring 4+3+2 cm, the heart of twin A measuring +1½+2 cm. Examination of the ventricular region revealed extensive fusion of the ventricular walls—a shallow groove on the surface indicating the junctional region. The ventricular cavities of A and B were in fact separate a thick muscular partition separating the two.

The inter ventricular septa of A and B were also thick and muscular but each presented a large crescentic defect. Examinatio of the atrial ca ities of A and B showed communication between the circulatory systems inasmuch as a large defect was present between the right atrial cavity of twin B and the left atrial cavity of twin A.

The aortic pulmonary mitral and tricuspid valves with their cusps had developed normally in each heart. The aortic and pulmonary trunks were normally disposed in each twin. A normally disposed inferior vena cava of twin B received the blood from the common liver mass. To the left of the aorta of twin A a vein of the same size as the inferior vena cava of twin B was present. This vein had no connection with the liver or the heart and was found to originate from smaller eins one from each lung of twin A. At the level of the aortic bifurcation it divided into two normal iliac veins. The pulmonary eins of twin B showed no malformations.

Urogenital syst m. In each, the kidneys showed the normal fetal lobulation with a single adrenal capping each kidney. No other adrenal tissue was found. The ureters the bladder uterus, uterine tubes and ovaries had developed normally.

Thymus. Both were of normal fetal type.

Central nervous system. Each brain weighed 350 grams and howed no malformation. Normally developed gyri and sulci together with a normal distribution of the grey and white nervous matter was found.

DISCUSSION

The exact incidence of human double monsters cannot be ascertained as their occurrence is not recorded particularly accurately Ripman (3) believes that the incidence is 1 in 50 000 for double monsters born in the last trimester El-Minawi et al (1) in a review of the literature report an incidence varying from 1 in 50 000 deliveries to 1 in 80 000 In 1954 Jorgensen (2) reported two cases of thoracopagus occurring in the same department within some weeks one of them even in a triplet pregnancy In our case we are unable to calculate any exact incidence

The aetiology of the phenomenon is unknown Some speculations as to the aetiology have recently been outlined (4)

The diagnosis of conjoined twins is a matter of considerable difficulty In our case the diagnosis was first made when obstruction to delivery was met in the second stage Out of 28 cases of double monsters (3) only 4 were diagnosed before the onset of labour and the majority were diagnosed when complications developed during labour From the X ray in our case a firm diagnosis of conjoined twins is not possible as no bony union is present in the monster Only the fact that the heads are at the same *level might suggest this very rare phenomenon* The passage of a hand up the birthcanal when labour was obstructed in the second stage gave the exact diagnosis in this as in most other cases of conjoined twins.

As for the treatment it was evident that the monster reported would be incapable of survival because of the large eventration and omphalocele So the choice of management was made under consideration for the mother A decision was made to deliver vaginally and then cervical perforation and traction were performed

REFERENCES

1 El-Minawi M F Shaaban H Nagrib Y El Sadek M & Shaaban, A Human double monsters. *Int J Gynecol Obstet 8* 648–652, 1970
2. Jorgensen G To tilfælde af Thoracopagus. Ugeskr Læg *116* 708–11 1954
3 *Ripman J A Conjoined twins as an obstetric problem Guy Hosp Rep 107* 173–184 1958.
4 Vestergaard P Triplets pregnancy with a normal foetus and a dicephalus dibrachius sirenomelus. Acta Obstet Gynecol Scand *51* 93–94 1972.

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Acta Obstet Gynec Scand 53 383–385 1974

RETROGRADE CERVICAL PERFORATION BY THE COPPER T DEVICE

Karl-Gösta Nygren and Elof D B Johansson

From the Department of Obstetrics and Gynaecology (Head Prof C Gemzell), University of Uppsala Uppsala S eden

Abstract. T cases of silent retrograde perforation of the cervi ere found after approximately 1200 inser tions of the contraceptive intra-uterine Copper T device. One perforation occurred after post-menstrual inser tion, the other after an immediate post-abortal inser tion. No irritation of the cervical tissue was found and the perforations seemed to heal quickly after the re moval of the devices.

Perforation of the uterus *during* the insertion of a contraceptive intra-uterine device (IUD) usually occurs through the posterior wall of the isthmic portion of an anteflexed uterus. Perforation of the uterus *after* the insertion has been studied by X-ray techniques revealing perforation or penetration into the uterine tissue. Bilateral partial perforation by the horizontal arms of a Copper T device into the isthmic part of the uterus associated with downward displacement of the device has been reported (1). Penetration or suspected penetration of the arms of the Copper T with the device *in situ* in the uterine cavity has also been described (3)

After the insertion of about 1200 Copper T devices (model TCu 200) we have found two cases of retrograde cervical perforation through the cervical tissue with the tip of the shaft of the T protruding into the pper part of the vagina.



Fig. 1. Case 1 0-gravida. Postmenstrual insertion. Finding at routine inspection 3 months after insertion of Copper T device. The tip of the shaft of the T has perforated the cervix below the cervical canal. In the photograph it is reflected in the speculum

CASE REPORTS

Case 1

Null-gravida, 25 years old healthy with regular menstrual cycles with a length of about 40 days Pelvic ex amination befor the insertion revealed no pathological findings. The distance from the fundus of the uterus to the external cervical os was 6 cm The insertion of the Copper T device was performed without difficulty or discomfort to the woman She returned after 3 months for routine examination She reported no adverse ef fects of the IUD. The tip of the shaft of the T was found to have perforated the cervix and was visible below the cervical os, as show in Fig 1 The threads hich are attached to the device near the end of the shaft, wer not sible The device wa easily removed by first pushing it into the uterine cavity and then pulling it out by means of curved forceps introduced through the cervical canal Three weeks later the woman was examined again and no sign of the perforation w found.

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53-742964



Fig 2 Case 2 II-gravida 0-para. Post-abortal insertion Finding at routine inspection 3 weeks after insertion of a Copper T device The tip of the shaft of the T has perforated the cervix to the right of the cervical canal The white piece in the cervical os is scar tissue and not a part of the T-device

Case 2

Nulli-para, 30 years old with a history of one uneventful spontaneous abortion and one severe episode of salpingitis She was admitted to our department for legal abortion in the 12th week of pregnancy The abortion was performed under general anaesthesia by cervical dilatation to Hegar 12, followed by vacuum curettage during concomitant infusion of oxytocin A Copper T device was introduced into the uterine cavity immediately after the curettage, while the patient was still under general anaesthesia. The abortion was uncomplicated. Three weeks later the patient returned for a scheduled routine examination and reported no complaints. Pelvic examination revealed the tip of the T to be located laterally to the cervical os as shown in Fig. 2. The threads of the device were hanging out from the tip of the shaft of the T into the upper part of the vagina When touched, the device could easily be brought into the cervical canal thereby visualizing a rift in the cervical tissue (Fig. 3) The device was removed without difficulty After 3 weeks no sign of the rift was found at pelvic examination.

DISCUSSION

Cervical perforation of the same type as described in this communication has previously been found for other types of IUDs (Tatum personal communication Nilsson personal communication). As shown in this study they do occur even with the Copper T device In the nulli-gravida woman (Case 1) a downward displacement from a comparatively small anteflexed uterus seems to have caused the perforation After the postabortal insertion (Case 2) a true perforation probably did not occur The threads were hanging out into the vagina from the tip of the device and it seems more likely therefore that they became imbedded in the cervical tissue when a small rift caused by the cervical dilatation preceding the curettage started to heal. The device then probably moved down through this artificial canal

Neither of the women in this report experienced any discomfort The abnormal position of their IUD was found on routine examination The perforation did not cause any detectable irritation of the tissues and the artificial canal through the cervix appeared to heal quickly after the removal of the IUD There seems to be no reason to expect any sequelae Therefore this rare and symptomless type of perforation of the cervix should not impose any limitation of the use of the Copper T device However the findings reported here support our opinion that a routine examination is advisable within the first few months after the insertion of an IUD



Fig 3 Case 2. A cervical rift was visualized when the device was brought into the cervical canal by light sideways pressure

ACKNOWLEDGEMENTS

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REFERENCES

1. Lehfeldt, H. & Wan, L. S.: Unusual uterine perforation with a new intrauterine device. Obstet Gynecol 37: 826, 1971
2. Nygren, K. G. & Johansson, E. D. B.: Insertion of the endo-uterine Copper T (TCu 200) immediately after first trimester legal abortion. Contraception 7: 299, 1973
3. Timonen, H., Luukkainen, T., Rakalo, J. & Tatum, H. J.: Hysterographic studies with the Copper T (TCu 200) in situ. Contraception 6: 313, 1973

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ANNOUNCEMENTS

An International Conference on the Human Semen & Fertility Regulation in the male will be held on April 24–26 1975 in Gordon Scott Hall, Wayne State University School of Medicine Detroit Michigan USA. Those, interested in presenting research papers should request forms for Expanded Abstracts. Those interested in attending the conference, should request "Preregistration Forms". Write to the Program Chairman Dr E. S. E. Hafez, Department of Obstetrics & Gynecology Wayne State University School of Medicine 550 E. Canfield Detroit, Michigan 48201 USA. Deadline for applications is Dec. 31 1974.

SUCCESSFUL QUADRUPLET PREGNANCY FOLLOWING OVULATION INDUCED WITH HUMAN MENOPAUSAL GONADOTROPIN AND HUMAN CHORIONIC GONADOTROPIN

Niels H. Lauersen, Myron Buchman and Carl G. Beling

From the Department of Obstetrics and Gynecology (Head Prof F Fuchs) The New York Hospital Cornell University Medical College New York USA

Abstract Induction of ovulation in anovulatory patients with gonadotropins has significantly increased the incidence of multiple gestations. A quadruplet gestation that occurred after induction of ovulation with human menopausal gonadotropin (HMG) and human chorionic gonadotropin (HCG) is described. All infants have survived, and have developed normally. The management of multiple gestations creates special problems for the Obstetrician. Early diagnosis and correct management with complete bedrest and hospitalization of the patient are essential for successful outcome. Control of the patient with urinary estriols, ultrasound and daily vital signs are most helpful. Organization of an alert perinatal staff composed of Obstetricians, Pediatricians, and Anesthesiologist contributes to fetal salvage. Problems during the antepartum and intrapartum care are discussed.

Multiple births have always created a great public interest (10). The birth of the Dionne quintuplets (2) in May 1934 stimulated enormous interest since no single quintuplets had previously survived more than 50 days and because of the early skillful medical care and the successful outcome.

A mathematical relation between the various cases of multiple births was first stated by Hellin (9) who reported that twin gestations occurred once in every 89 births, triplets once in 89^2 and quadruplets once in 89^3 births.

Guttmacher (7) analyzed the occurrence of twins and triplets among more than 57 million births reported by the United States National Office of Vital Statistics during the period 1928 to 1949. Twins occurred in 1.108% (1:89.3) of all births and triplets occurred in 0.018% (1:9130). Guttmacher also found a racial difference in regard to multiple births. The mean frequency of twinning among 50 million white births in the United States was 1 in 92.4 deliveries whereas among 7 million blacks it was 1 in 73.8 deliveries. The difference in regard to triplets was even more striking.

With the introduction of ovulation-inducing drugs the incidence of multiple gestations has increased significantly. One of the main complications of gonadotropin treatment is ovarian hyperstimulation leading to multiple conceptions. In several series of induction of ovulation with gonadotropins, the multiple gestation rate has been reported to be 30–50% (6, 1, 8, 15, 16, 12).

One case of quadruplet pregnancy following ovulation induced with human menopausal gonadotropins (HMG) (Menotropin (Pergonal) Cutter Laboratories, Berkeley, California) and human chorionic gonadotropins (HCG) (Ayerst Laboratories, New York, N.Y.) which was successfully managed and delivered at The New York Hospital-Cornell Medical Center is reported.

CASE REPORT

L. S. was   -year-old white female. Menarche occurred at age 14; her periods had always been extremely irregular with only 3 menstruations a year. She was first seen seven years ago in 1966 for contraceptive advice and given mestranol norethindrone 1/50 21. The patient remained on the oral contraceptives for about one year after which time she tried to conceive. She was a very slender woman overly concerned with her weight and her oligomenorrhea was felt to be related to

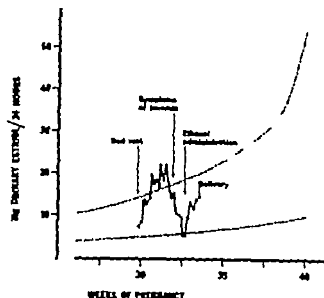


Fig 1 Daily determination of estriol from the day the patient was admitted to the day before delivery The upper and lower dashed lines are 95% confidence limits of the estriol excretion during normal pregnancy

her periodic strict dieting with weight loss often in the range of 15–20 pounds

Four years ago she underwent a routine infertility work-up with entirely normal anatomical findings Her physical and pelvic examination were normal the basal body temperature curve (BBT) was flat she did not respond to progesterone with withdrawal bleeding and vaginal smears revealed low estrogen level

She was then treated with estradiol valerate followed by hydroxyprogesterone caproate and was given clomiphene citrate (Clomid William S Merrell Co Division Richardson Merrell Inc Cincinnati, Ohio) after the withdrawal bleeding She conceived after several courses of clomiphene citrate raising the doses from 50 mg daily for 5 days to 100 mg for 5 days During the 27th week of the pregnancy she developed severe epistaxis and was admitted to New York Hospital where she was given 5 blood transfusions She subsequently had an uneventful delivery in 1969 of a ?900 g healthy female infant She breast-fed the baby for 4 months.

Following the delivery she did not menstruate for 8 months when she again was treated with estrogen and progesterone She wanted to conceive again but was unsuccessful even after several courses of Clomid Her BBT curve was still flat and the estrogen level remained low It was therefore decided to induce ovulation with HMG-HCG therapy

She was given Pergonal for one cycle after withdrawal bleeding was induced One ampoule HMG containing 75 I U of FSH and 75 I U of LH was given daily for 7 days followed by ? ampoules daily for 4 days at which time she was found to have 3+ to 4+ cervical mucus with good ferning and a high estrogenic index in the vaginal smear HCG 2500 I U was then given intramuscularly on two consecutive days to which she responded with ovulation and conception

She developed considerable nausea and vomiting and rapid enlargement of her uterus Multiple pregnancy was suspected because the uterus was significantly larger than expected this was confirmed by sonogram which revealed 4 fetuses at 18-week gestation She again developed severe epistaxis during this pregnancy and was in her 17th week given three units of packed red cells.

The patient was very intelligent and cooperative. She remained on bedrest at home In her 28th week of gestation she developed upper respiratory infection and this in combination with the enormous distention of her abdomen resulted in hospitalization at 28 $\frac{1}{2}$ weeks gestation She was admitted to the Eugene F Du Bois Research Pavilion at the New York Hospital

While in the hospital she was confined to bedrest and given high protein diet with salt restriction. She was followed with daily urinary estriols weekly sonograms (3) and daily weight control There was a good initial response to the hospitalization and bedrest with increasing urinary estriols from 8 3 mg/24 hr (3 μg/ml) at admission to 20–22 mg/24 hr at 31–32nd weeks At 32 $\frac{1}{2}$ weeks there was a sudden drop in the urinary estriol levels to 5.5–5 4 mg/24 hr (3 8 to 2 3 μg/ml) (Fig. 1) This was associated with the development of the symptoms of mild toxemia She developed 2+ to 3+ ankle edema and had excessive weight gain The blood pressure however remained stable around 110–120/70–80 and there was no proteinuria The serum electrolytes remained normal but the Blood Urea Nitrogen increased from 8 to 20 mg% the uric acid was 7 7 mg% and serum albumin had dropped to 3 0 g% (normal range 3 3–4 7 g%) The patient had difficulty eating due to the extreme extension of the uterus She was therefore given 3 units of salt-poor albumin intravenously

th good diuretic effect and there was no further weight gain during the next four days.

The patient began to complain about increasing frequency of Braxton Hicks contractions and increasing discomfort with back pain. She was then given Ethanol orally (5) mixed with fruit juice ad lib with some uterine relaxing effect. There was at the same time some recovery in the urinary estriol levels which increased to 13 7 mg/24 hr (8.1 μg/ml) the day prior to delivery (Fig. 1). At 33 ⁴⁄₇ weeks gestation, she complained one evening about increased discomfort she had difficulty in finding a comfortable position and had increased uterine activity At 6.30 a.m. the next morning, the membranes of the first amniotic sac suddenly ruptured spontaneously and she was immediately taken to the Delivery Floor On examination the cervix was fully dilated the first fetus was in a vertex presentation with the leading point at the spines. She was immediately taken to the delivery room where in a few minutes an alert staff comprising 4 obstetricians 3 pediatricians 2 anesthesiologists and several obstetrical nurses were present. The patient was acquainted with the Lamaze method and wanted to be awake during the procedure This was permitted since no complications occurred The delivery went smoothly and no anesthesia was required

The first baby—a boy 1960 g, Apgar 9 was delivered spontaneously 54 min after the rupture of the membrane The second fetus a girl 1920 g was in compound presentation. The right arm was coming down alongside the head and it was possible to lift the baby up because of intact membranes and to bring the baby head down into the pelvis The head was in an occiput posterior position and was brought down further by the well directed suprapubic pressure When the vertex was in the midpelvis a Simpson forceps was applied and the baby was delivered in the direct occiput posterior position an Apgar score of 8 was recorded

The third fetus was in transverse position internal version and breech extraction was performed and female infant of 1880 g, Apgar score 6, was delivered The fourth fetus was also in transverse position internal version and breech extraction was done and a female infant 1760 g, Apgar score 5 was delivered The two last infants required active resuscitation 10 ml of bicarbonate and oxygen administration. The placentas were delivered spontaneously the uterus was explored and found intact. Intravenous oxytocin was given and the uterus contracted well. The post-partum course was benign and the patient was discharged on the 6th post-partum day All the infants went to the Premature Nursery where they all did well with no signs of respiratory distress syndrome. The patient breast-fed all the babies and they were discharged at 3 weeks of age. Examination of the placentas revealed four separate placentas indicating that the quadruplets developed from four ova.

DISCUSSION

The incidence of multiple pregnancy has increased significantly since the introduction of gonadotropin treatment to anovulatory infertile women. One of the main problems during ovulation induction with HMG-HCG is ovarian hyperstimulation and multiple ovulation. While the ovarian hyperstimulation syndrome can be prevented in most instances with careful monitoring of urinary estrogens the majority of the authors feel that there are no means to control the number of ova released from the ovaries at the time of ovulation (6 12 4). Gemzell & Roos (6) found that following the first treatment with HMG a woman with a long period of amenorrhea and some pituitary gonadotropic function runs a greater risk of multiple births than those women who have no gonadotropic function and have been treated several times. They feel therefore that conception following the first course of treatment is undesirable Insler et al (11) have stated that it is adequate to follow a cervical mucus scoring system in patients with amenorrhea associated with low endogenous gonadotropin levels. Karam et al. (13) feel that ovarian hyperstimulation and multiple pregnancy of triplets or more can be sharply reduced if not eliminated by monitoring the urinary estrogens

If multiple pregnancy occurs an early diagnosis is very important to improve fetal salvage. The best method of early diagnosis is by sonography which can safely be done as early as 10–12 weeks gestation (3) Ultrasonography furthermore is helpful in determining fetal growth and placental location. The main concern of multiple pregnancy is prevention of premature labor and toxemia. Early complete bedrest is therefore mandatory It is important to have close contact with the family and

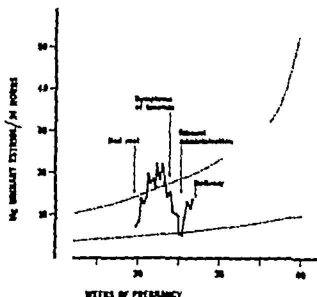


Fig 1 Daily determination of estriol from the day the patient was admitted to the day before delivery The upper and lower dashed lines are 95% confidence limits of the estriol excretion during normal pregnancy

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Jewelewicz, R. James, S. L., Plaster M Dyrenfurth I Warren, M. P & Van de Wiele R. L. Quadruplet gestation after ovulation induction with menopausal gonadotropins and pituitary luteinizing hormone. Obstet Gynecol *40* 1 1972.

Kuran, K. S. Taymor M L. & Berger M J Estrogen monitoring and the prevention of ovarian overstimulation during gonadotropin therapy Amer J Obstet Gynecol *115* 972, 1973

Lauersen, N H. & Fuchs, F Behandlung von drohender Frühgeburt mit Alkoholinfusion. (Treatment of premature labor with intravenous alcohol administration) Perinatale Medizin, 3 Deutscher Kongress für Perinatale Medizin, Berlin, November 26–28, 1970, Georg Thieme Verlag, Stuttgart, p 87

Nesworth, R. S., Todd W D Turksoy R. N & Van de Wiele R L. Successful quadruplet pregnancy in patient treated with human menopausal gonadotropins. Obstet Gynecol *40* 1 1972.

16. Turksoy R N Toy B L., Rogers J et al. Birth of septuplets following human gonadotropin administration in Chiary-Frommel syndrome. Obstet Gynecol *30* 692, 1967

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to impress upon them the problems that can occur if strict bedrest is not carried out Hospitalization about ?7th to 28th weeks is essential We were fortunate that our patient could be admitted to the research ward at the New York Hospital the Eugene F Du Bois Pavilion however if no re search facilities are available the patient should be admitted to a floor with a competent staff able to handle high risk patients or else the patient should be transferred to an institution with such facilities

In reviewing the reported cases of multiple pregnancies (1 6 8 1? 15 16) it is interesting to observe that all patients developed pre-eclampsia and delivered prematurely Our patient developed toxemia around the 33rd week in spite of all pos sible precautions such as strict bedrest high pro tein intake and sedatives The patient s ability to maintain an adequate oral intake decreases usually because of the enormous distention of the uterus and intravenous feeding is occasionally necessary The serum albumin in the described patient dropped significantly in combination with the development of pre-eclampsia. However the patient responded well to administration of intravenous albumin with good diuresis

The hospitalization of the patients permits the early diagnosis of threatened premature labor and makes it possible to initiate treatment immediately Intravenous infusion of ethanol as described by Fuchs et al (5) has proven useful in many cases of multiple pregnancies (14) The treatment was useful in a recent case of sextuplets in Denver Colorado (Engle personal communication) In our case the patient was given ethanol orally but this did not prevent rupture of the membranes In hind-sight the patient might have been treated with ethanol i v when she noted increasing uterine ac tivity the night before.

Premature rupture of the membranes often initiates parturition in multiple gestation and once this has occurred attempts to stop labor are almost always unsuccessful and may not be justified The first infant is often delivered very rapidly and it is therefore important to have an alert staff of obstetricians anesthesiologists and pediatricians A premature nursery must be available and adequately prepared to handle four to five premature infants at one time. The delivery should be rapid and atraumatic. The patient should be given an intravenous infusion and sufficient blood must be available The infants are usually small and vaginal delivery can generally be performed General anesthesia must be available if uterine relaxation becomes necessary in order to perform internal versions. If the delivery does not proceed smoothly delivery with cesarean section may become necessary The patient must be carefully observed for post partum hemorrhage and should be followed in the recovery room for at least 24 hours post partum

Birth of quadruplets quintuplets and sextuplets are often treated as sensations by the news media. However if the patient and her family are against this kind of publicity it is the obligation of the hospital to avoid it

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REFERENCES

1 Aiken R A An account of the Birmingham "sex tuplets J Obstet Gynaecol Br Comm 76 684, 1969
2. Dafoe A R Dionne quintuplets. JAMA 103 673 1934
3 Donald I Practical Obstetrical Problems 4th ed p 934 J B Lippincott Co Philadelphia.
4 Engel T Jewelewicz R Dyrenfurth I Speroff, L. & Van de Wiele R L. Ovarian hyperstimulation syndrome Am J Obstet Gynecol 112 1052, 1972
5 Fuchs F Fuchs, A R Poblete V F Jr & Risk, A Effect of alcohol on threatened premature labor Amer J Obstet Gynecol 99 627 1967
6 Gemzell C & Roos, P Pregnancies following treatment with human gonadotropins with special reference to the problem of multiple birth Am J Obstet Gynecol 94 490 1966
7 Guttmacher A F The incidence of multiple birth in man and some of the other unipara Obstet Gynecol 2 2 1953
8 Hack, M Brish M Serr D M Insler V & Lunenfeld B Outcome of pregnancy after induced ovulation JAMA 211 791 1970
9 Hellin D Die Ursache der Multiparität der uniparen Tiere uberhaupt und der Zwillings-Schwangerschaft beim Menschen 70 pp Seitz & Schauer Munich 1895
10 Hellmann, L. M & Pritchard J M Williams Obstetrics 14th ed Appleton Century Crofts N York 1971
11 Insler V Melmed H Eden E Serr D & Lunenfeld B In Clinical Application of Human Gonadotropins (ed G Bettendorf and V Insler p 87 Georg Thieme Verlag, Stuttgart, 1970.